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STUDIES IN TISSUE RESPIRATION AND ENDOCRINE FUNCTIONS

I. The Influence of Thyroidectomy on the Indophenol Oxidase Content of Animal Tissues

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It is a matter of general knowledge that hypothyroidism in mammals, whether due to disease or experimental removal of the glands, results in a condition of subnormal body temperature, imperfect heat regulation and very marked changes in body metabolism. Although a wealth of literature has accumulated upon these subjects during the last two decades, our knowledge regarding tissue respiration under the same physiological conditions is comparatively meager. The circulatory disturbances which might limit the supply of oxygen to the tissues in the intact animal, the actual capacity of the isolated tissues to utilize oxygen and the oxidase systems involved are in need of further study.

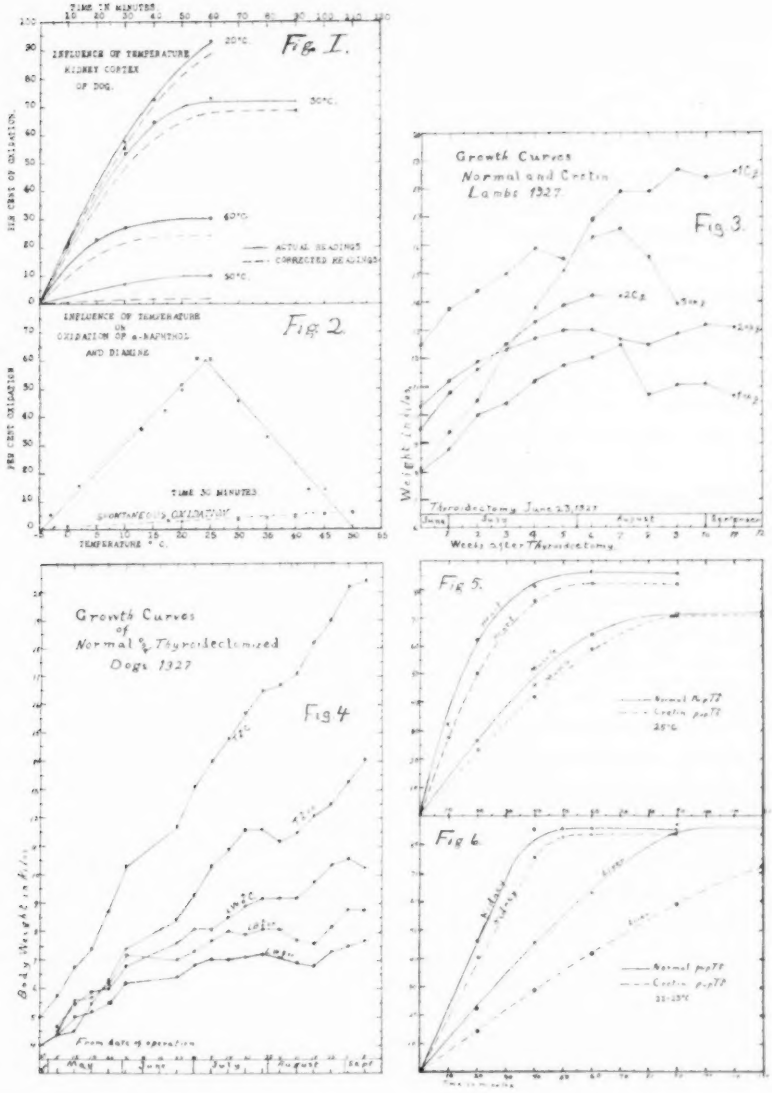
The factors concerned in maintaining physiological oxidations and body metabolism at the normal level are at present only partially understood. Grafe, Reinwein and Singer (1925) have shown that the constant relation between metabolism and body surface area is a function of the intact animal. On the assumption that activity of the oxidase systems and regulation of the gaseous exchange might play a very important rôle in maintaining the normal body metabolism of an organism, a series of experiments has been planned with the hope of gathering further data regarding the nature of these fundamental physiological processes. The present paper, although complete in itself, deals with one phase of the subject only, as revealed by a single method. Further studies of these problems are now in progress.

Methods. The oxidizing power of the tissues from normal and thyroidectomized animals was studied quantitatively by estimating colorimetrically the amount of indophenol blue formed in a given time from the

oxidation of a substrate composed of a-naphthol and dimethyl-paraphenylene-diamine-hydrochloride in alkaline solution (Dye, 1927). The laws governing the reaction have been reported by Vernon (1911). Oxidation of this substrate occurs spontaneously at a very slow rate when the reacting substances are exposed to air, but may be accelerated twenty times or more when minced tissue is added. One gram of sand-tissue mixture was accurately weighed out in a perfectly flat-bottomed Petri dish (9 cm. diameter), after which 5 cc. of the substrate were added, the contents mixed thoroughly and allowed to stand for any required interval at room temperature. Since the temperature of the laboratory varied between 22°C. and 25°C. from day to day, more reliable results were obtained when the determinations for any given tissue were run simultaneously for the control and experimental animals. This eliminated not only the factor of temperature, but also that of light.

Comparisons can, therefore, be made with exactness only between the results from the tissues of normal and thyroidectomized animals of a single experiment and between those of different groups only in a general way. Since, as will be shown below, temperatures between 22°C. and 25°C. have very little effect on the course and velocity of the reaction, even such comparisons become very instructive and reliable. Other factors being equal, the results were, within limits of error, quite constant from animal to animal. Although Vernon (1911) found absolute darkness to diminish the spontaneous formation of indophenol blue to less than 80 per cent of that formed in the presence of light, it was without influence on the same reaction when catalyzed by tissue.

EXPERIMENTAL OBSERVATION AND DISCUSSION. *Temperature.* Since it is well known that thyroidless animals have a subnormal body temperature, it is fundamentally important to know what effect this would have on the oxidation processes in the tissues of such an animal. Furthermore, it was found essential that a series of experiments be made to determine the influence of temperature on the course and velocity of the indophenol oxidase reaction under the conditions of the present investigation. The first problem is beset with certain quite uncontrollable obstacles. In the first place, since the activity of the reducing substances is proceeding hand in hand with that of the oxidase systems and is able to exert its reducing power upon the end-products of the oxidation reaction, these two processes tend to neutralize each other. Under certain conditions oxidation may be completely masked by the reductions occurring simultaneously. Hence the inability of a given tissue to form indophenol blue under a given set of conditions cannot be taken as evidence of the absence of the oxidase unless these are ideal for such a reaction. Temperature seems to be a factor which has a most striking influence in determining which of these processes gains the ascendency.



In figure 1, representative curves are given to show the influence of temperature on the course and velocity of indophenol blue formation, both spontaneously and as catalyzed by animal tissue. These determinations were carried out by placing the Petri dishes containing the substrate alone

or substrate plus one gram of sand-tissue mixture in an incubator or refrigerator, the temperature of which could be maintained constant for any length of time. They were always kept in complete darkness except when the doors were opened to stir the contents of the plate. Under these conditions and with the concentration of substrate used, spontaneous conversion of a-naphthol and the diamine into indophenol was proportional to the temperature, i.e., a direct linear proportinality was established, as can be seen from table 1 and figure 2. Each test was of exactly thirty minutes duration.

It will be seen from figure 2 that, under the conditions of these tests, the optimum temperature for the indophenol oxidase system is between 23 and 24°C., that at a temperature of 0°C. an appreciable amount of oxidase activity exists, while at a temperature corresponding to that of the living animal (37°C.) its activity as measured was greatly reduced. These conclusions were drawn from numerous tests on heart, kidney, liver and skeletal muscle from thirteen guinea pigs and two dogs. When the tissue is held at 50 to 55°C. for thirty minutes and subsequently tested for its ability to oxidize the substrate, or when the tissue-substrate mixture is placed immediately into the incubator at the same temperature, little or

TABLE 1

Influence of temperature on the spontaneous formation of indophenol

Temperature, °C.....	−3	0	17	19.5	20	30	33	40	42	44	45	50
Per cent oxidation.....	1	1.9	3.25	2.14	2.5	3.3	3.6	4.3	4.5	5.1	5.6	5.5

no formation of indophenol is visible, in fact, that which is formed spontaneously is rapidly reduced. Obviously the reducing power of the tissues is not destroyed while the oxidase is completely inactivated at 50°C. in thirty minutes.

Vernon (1911) believes that the action of indophenol oxidase is accelerated by temperature in the same way as that of the other enzymes, but it is so unstable at high temperatures that its activity quickly ceases. It is highly probable that the reduction processes at temperatures above 23°C. mask the feebler oxidations. In support of this conclusion, the work of Harris (1911) upon the reducing endo-enzymes of liver and kidney shows that the reaction is distinctly accelerated by a rise of temperature from 15°C. to 46°C., completely inhibited below 15°C., progressively weakened at temperatures above 60°C., and finally destroyed at 75–80°C. Herter (1905) found a marked acceleration of reduction in fever and in tissues in vitro at 43°C. On the other hand, he showed that a fall of body temperature to 30°C. was attended by a diminished reduction of methylene blue intravenously injected (1904).

The curves shown in figures 1 and 2 are typical for all tissues and for all animals studied, i.e., the optimum temperature is always between 23 to 24°C.; the minimum, beyond which no oxidation processes occur, between −5°C. and −12°C.; while the maximum or destruction temperature is between

TABLE 2

Lambs of the same number are twins, and pups of the same initial letter are from the same litter. Those from each group designated "op" were thyroidectomized

ANIMAL	DATE	THYROIDECTOMY WEIGHT	AGE	KILLED	REMARKS
		kgm.	*weeks*		
Lamb, 1 ♀ C		8.1	4	9/ 9/27	Treated for stomach worms 8/25/27
Lamb, 1 ♀ op.	6/16/27	8.0	4	9/ 9/27	Stunted, typically cretinoid
Lamb, 2 ♀ op.	6/16/27	10.3	6	9/ 8/27	8/19/27, weak and losing weight. Treated for stomach worms 8/25/27
Lamb, 3 ♀ op.	6/16/27	12.5	7	8/25/27	8/25/27, very weak and had been losing weight for some time
Lamb, 2 ♀ C		9.5	6		Died 8/13/27; cause unknown
Pup, T ♂ C		5.0	10	9/12/27	Collie pup
Pup, T ♂ op.	4/27/27	4.0	10	9/12/27	Collie pup
Pup, LW ♂ C.		4.65	10	9/17/27	Mongrel. Small breed
Pup, LB ♂ op.	5/ 5/27	4.58	10	9/17/27	Mongrel. Small breed. Typically cretinoid
Pup, LW ♀ op.	5/ 5/27	4.35	10	9/17/27	Mongrel. Small breed. Typically cretinoid

Each of the experimental groups was properly controlled as to sex, age and experimental treatment. Recovery from the immediate effects of the operation was prompt, the wound healed by first intention. After the first two weeks the pups were given perfect freedom in an open enclosure with proper shelter and food. The food consisted of dog biscuits and milk. Two weeks after thyroidectomy all the puppies developed mange. After four treatments with sulphur-lime at weekly intervals, a complete cure was effected. There were two pairs of twin lambs and an odd one. The smaller of each pair was kept as a control. They were kept in an open pasture with ample shelter and were permitted to suckle the ewes.

During the last few weeks before killing the thyroidectomized lambs became very weak and exhibited all the signs of cretinism in a marked degree. These have been reported for sheep by Simpson (1913, 1924). Great care was necessary to keep them alive and thyroxin medication would have been imperative were it necessary to prolong the experimental period for any length of time.

50–55°C. Obviously the time factor is important, for a tissue subjected to 40°C. may be inactivated through longer exposure. The steepness of the velocity and quantity gradients for other tissues, as might be shown by similar curves, diminish in the following order: heart, kidney, liver and

muscle. At 20°C. the velocity of the oxidation reaction is practically unaffected. It is at first linear with the time and until the amount of available substrate begins to fall below the optimum, does not begin to lag. Soon after this condition is reached a state of equilibrium tends to be established, but this is not complete at the end of one hour when 90 per cent of the available substrate has been oxidized. At 40°C. equilibrium is reached when about 70 per cent of the indophenol blue is formed and the reaction begins to subside at the end of 30 to 40 minutes. At higher temperatures equilibrium is reached when less than 30 per cent for 40°C. and less than 10 per cent for 50°C. is oxidized, while the reaction begins to fall off almost at once. It would seem that two factors are responsible for these

TABLE 3

	ANIMAL								
	Lamb 1 ♀ C.	Lamb 1 ♀ Op.	Lamb 2 ♀ Op.	Lamb 3 ♀ Op.	Pup T ♂ C.	Pup T ♂ Op.	Pup LW ♂ C.	Pup LB ♂ Op.	Pup LW ♀ Op.
Weight in kilos at thyroidectomy	8.1	8.0	10.3	12.5	5.0	4.0	4.65	4.58	4.35
Weight in kilos when killed	18.6	10.7	13.1	13.9	20.4	14.1	10.3	8.8	7.7
Maximum increment of body weight during experiment	10.5	4.5	2.9	4.1	15.4	10.1	5.95	3.22	3.35
Increment of body weight at last weighing	10.5	2.7	2.8	1.4	15.4	10.1	5.65	3.22	3.35
Maximum increment of body weight in per cent	130	56	28	33	308	252	128	92	77
Per cent increment of body weight at last weighing	130	34	27	11	308	252	122	92	77
Week after operation—maximum body weight	11	7	10	7	19	19	18	18	18

phenomena. First, with increasing temperatures the influence of the reducing substances is augmented at the expense of the oxidase, and secondly, especially at 40°C. and 50°C., there is a progressive inactivation of the oxidizing systems while the activity of the reducing agents is unaffected or even enhanced.

Thyroidectomy, growth and tissue oxidase. Ten animals were employed in these experiments, five lambs and five pups. In each case, two out of the five were kept as controls. A brief account of the protocols is given in table 2. Sufficient parathyroid tissue to prevent tetany was left behind in each of the animals from which the thyroids were removed. The fact that the external parathyroids are more caudally situated in the sheep makes this an easy matter in this animal. In the pups the two parathy-

roid glandules, which are usually visible, one beneath the capsule of each thyroid, were located and carefully left according to the method of Dot (1923). No animal of this series developed tetany. This indicated that sufficient parathyroid tissue remained functionally intact.

The exact and fundamental mechanism involved in the processes of growth is imperfectly understood, but the work of numerous investigators

TABLE 4

Indophenol oxidase and thyroidectomy

	LAMB															
TIME	1 ♀ Control				1 ♀ Operated				2 ♀ Operated				3 ♀ Operated			
	H	K	L	M	H	K	L	M	H	K	L	M	H	K	L	M
minutes																
20	61.0	28.2	18.8	9.5	47.4	15.7	15.0	3.7	45.0	21.6	23.1	6.4	62.8	9.0	32.4	4.2
40	87.7	57.1	34.7	17.9	74.0	36.1	28.0	6.1	83.3	45.9	49.1	9.2	73.5	19.8	66.0	9.5
50	87.7	72.9			76.0	48.9										
60	87.7	82.9	53.0	29.2	75.4	64.3	45.4	10.0	82.9	70.0	82.8	18.2	73.5	37.4	75.5	16.4
90	87.7	88.0	58.9	45.3	75.4	84.9	60.3	20.2	86.5	86.4	96.7	28.0	73.5	54.0	75.1	24.4
120			58.9	52.5			70.8	29.7			96.7	46.5			75.1	35.6

These values have been corrected for spontaneous oxidations of the substrate.

TABLE 5

Indophenol oxidase and thyroidectomy

	PUPS																			
TIME	T ♂ Control				T ♂ Operated				LW ♂ Control				LB ♂ Operated				LW ♀ Operated			
	H	K	L	M	H	K	L	M	H	K	L	M	H	K	L	M	H	K	L	M
minutes																				
10	32.0				27.4				37.1				26.9				31.6			
20	62.0	45.3	22.2	26.2	50.2	40.9	14.9	23.6	57.1	42.5	21.3	26.0	46.5	37.2	17.2	24.0	52.0	35.9	19.0	23.6
40	80.0	85.7	45.6	45.4	76.0	76.6	27.8	42.2	86.2	88.0	43.8	49.6	83.3	78.3	37.5	50.5	84.0	81.6	40.4	47.0
50		85.3				82.9					56.0									
60	86.5	85.0	63.0	64.1	82.2	83.3	41.5	58.8	81.9	89.7	67.0	65.6	82.6	87.3	50.6	62.8	84.0	89.2	55.3	61.3
90	85.1	84.6	87.4	71.5	81.6	83.7	58.7	71.5	81.9	89.7	77.4	79.2	83.3	88.0	71.6	80.6	83.3	89.2	71.6	79.2
120			83.8	70.5			72.1	71.5			88.6	80.5			86.8	89.6			86.8	79.8

These values have been corrected for spontaneous oxidation of the substrate.

(Uhlenhuth, 1919; Gudernatsch, 1912, 1913–14; Abderhalden, 1919; Cameron, Carmichael and Sedziak, 1921; Simpson, 1924, et al.) presents convincing evidence that the glands of internal secretion play a very important part in this respect. The close relationship which exists between the functions of the thyroid and general growth processes in mammals has been given special attention because of the relatively frequent occurrence of thyroid disorders in man.

Growth curves are given for each of the lambs in figure 3 and for each of the pups in figure 4, while a comparison of absolute and percentile increments in body weight, above that of the initial weighing, may be found in table 3. A lag in the growth curves was noticed after the first week with the lambs and after the second week with the pups. Age as a factor in affecting the growth and body weight of sheep after removal of the thyroids has been reported by Simpson (1913, 1924).

TABLE 6

Showing relative amounts of oxidation by tissue from normal and thyroidectomized lambs. The values obtained for the normal being taken as 100 per cent in each case

LAMB	TIME																			
	20 minutes				40 minutes				60 minutes				90 minutes				120 minutes			
	H	K	L	M	H	K	L	M	H	K	L	M	H	K	L	M	H	K	L	M
1 ♀ C.	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1 ♀ Op.	78	56	80	39	84	63	81	34	86	78	86	34	86	96	102	45			120	57
2 ♀ Op.	74	77	123	67	95	80	141	52	94	84	156	62	98	98	164	62			165	89
3 ♀ Op.	103	32	172	44	84	35	190	54	84	45	142	56	84	61	128	54			127	68

TABLE 7

Showing relative amounts of oxidation by tissue from normal and thyroidectomized pups. The values obtained for the normal being taken as 100 per cent in each case

PUPS	TIME																			
	20 minutes				40 minutes				60 minutes				90 minutes				120 minutes			
	H	K	L	M	H	K	L	M	H	K	L	M	H	K	L	M	H	K	L	M
T ♂ C.	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
T ♂ Op.	81	90	67	90	95	90	61	93	95	98	66	92	96	99	67	100			86	101
LW ♂ C.	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
LB ♂ Op.	81	88	81	92	97	89	86	102	101	97	76	96	101	98	92	102			98	100
LW ♀ Op.	91	84	90	91	97	93	92	95	102	99	83	93	102	99	92	100			98	99

Since growth is fundamentally dependent upon metabolism and both in turn probably paralleled by tissue oxidations, a diminution in the amount, activity, potency, or some other property of the oxidase systems may reasonably account for the low level of metabolism, subnormal temperature, disturbed heat regulation and stunted growth and reduced regenerative power of the tissue in thyroidless animals. That this state of things actually exists is supported by the present investigations on lambs and pups.

Indophenol oxidase of the tissues. The data from which this conclusion is drawn may be found in tables 4 and 5. Table 4 is a compilation of

actual oxidation values based on the percentage of complete oxidation of the substrate and which have been corrected for spontaneous oxidation. Readings for heart ventricle, kidney cortex, liver and skeletal muscle from the normal and thyroidectomized lambs are given for time intervals which vary from 20 to 120 minutes. Similar figures for the same tissues of normal and thyroidectomized puppies are recorded in table 5. In tables 6 and 7 these values are expressed on a percentage basis. The amount of oxidation occurring with any tissue of the normal control animal at any given time is taken as unity (100 per cent). The relative amount of oxidation for the same tissue and time interval for the thyroidectomized animals is expressed in terms of per cent of that occurring in the case of the normal. From the data presented in tables 4 and 5 it is possible to form velocity curves in which the per cent of oxidation is plotted against the time. Such curves have been drawn in each instance for purposes of analysis and study, all of which cannot be presented in this paper. Approximate curves can be seen at a glance from the values given in these tables. Those for kidney taken from experiment T with pups are shown in figures 5 and 6. These are offered as representative though they may differ from animal to animal and from tissue to tissue. More marked separation between the curves for normal and cretin animals might have been shown if those for lambs 1 ♀ C. and 1 ♀ op. had been chosen. This would indicate a greater reduction in oxidizing power with these animals. With few exceptions, the velocity of oxidation was greatly reduced in the tissues of the cretins, particularly in thyroidectomized lambs.

From these tables it is obvious that not all tissues have the same power to oxidize the substrate. It will be seen that 0.5 gram of normal heart muscle, with the quantity and strength of substrate used, is able to bring the oxidation to an equilibrium, when 80 to 90 per cent is oxidized, in about forty minutes. On the other hand, it requires about ninety minutes for liver and muscle to reach the point of equilibrium. Kidney is intermediate in its speed of action and at times may approach that for heart muscle. Just what factors might influence the equilibrium point were not determined, but it is probable that some of these at least may exert their influence through their action upon the reducing substances. The influence of temperature has already been mentioned. At any rate the point at which the reaction comes to an equilibrium is not always constant for the same tissues from different animals, even when the tests are run side by side under apparently identical conditions. The reaction is one of linear proportionality when the per cent of oxidation of a-naphthol and diamine is plotted against the time, until the amount of available unoxidized substrate becomes a limiting factor. At this point the velocity rapidly falls until equilibrium is established.

From tables 6 and 7 it will be seen that the percentile values for any

given tissue of the cretin animals approach those for the same tissue of the control, against which they are compared, as one passes from left to right along the columns, or to be more exact, as the time of the oxidation period is prolonged. This is obviously the result of allowing sufficient time for the slower oxidations catalyzed by the tissues of the thyroidectomized animals to overtake the faster reactions of the controls. This fact is clearly illustrated by the curves for liver in figure 6. The liver curve for the cretin would doubtless reach an equilibrium with that of the normal in approximately two and one-half hours. The reaction is thus quantitatively, but not qualitatively altered.

A word of explanation may be offered for the high percentile values given in the case of liver from the thyroidectomized lambs. It was noticed at the time of running these tests that these values for this tissue of the normal animal were unusually low as compared with average figures obtained, and since the percentile values are based upon these, they appear proportionately large.

If the velocity of oxidation as catalyzed by the normal tissues of lamb 1 ♀ C is compared with that for similar tissues from pups T ♂ C and LW ♂ C, it will be noted that they are approximately the same, that for the pups being slightly greater. On the other hand, there is a marked difference between the velocities for those of cretin lambs and cretin pups. In the latter, these are distinctly lower for liver, while for kidney and skeletal muscle the reduction in velocity is much more prominent in the lambs, that for heart is only slightly lower in these animals. The percentile values for these animals would indicate that heart tissue maintains its integrity, as measured by its power to catalyze the oxidation of the substrate, longer than the other tissues. In lamb 3 ♀ op. this power in kidney and skeletal muscle is greatly reduced while heart, probably the most vital organ studied, still retains approximately 84 per cent of its normal oxidase activity. The same is true for lamb 1 ♀ op. while it is not so obvious in the case of 2 ♀ op. No such marked difference exists between the tissues of the cretin pups. In these the reduction in oxidizing power seems to affect all animals and tissues to about the same extent. This is probably due to the fact that the severity of the cretinoid condition was much less evident in the latter as judged from the symptoms. Age is probably a factor. The puppies were, on an average, twice as old as the lambs when thyroidectomized.

From the data presented, it is certain that the relative power of the tissues to catalyze the formation of indophenol is markedly reduced in experimental hypothyroidism in pups and lambs when compared with that of normal animals of the same sex and age. This reduction, as shown at the end of 20 minutes, is most marked in the skeletal muscle of the lambs where it amounts to 61 per cent in 1 op., 33 per cent in 2 op. and 56 per cent in

3 op., or an average of about 50 per cent. The average for kidney is lower (45 per cent), while that in heart and liver is approximately 24 per cent and 20 per cent respectively. A decrease of from 10 to 20 per cent was found in the same tissues of cretin pups 18 to 19 weeks after thyroidectomy, all tissues being affected to about the same extent. The mean of these percentages would agree favorably with the well-established fact that in hypothyroidism the basal metabolism may be reduced 30 to 40 per cent below normal (Kendall, 1919; Plummer, 1924).

That there is a diminution in the carbon dioxide output, especially when the external temperature is high, has been demonstrated by Hauri (1919) and others. Ample evidence has been submitted to show that this may be reduced to 30 per cent of the normal values (Weil, 1924). Associated with this fall in carbon dioxide production is an appreciable, but, according to Schenck (1922), smaller drop in oxygen consumption. This author further states that the metabolism is not only depressed, but qualitatively altered, the low R. Q. probably indicating a greater fat and protein metabolism. By the administration of thyroxin, metabolism may be restored and maintained over periods measured in years (Kendall, 1919).

Kendall (1919) has pointed out that the thyroid glands are not absolutely essential to life, but to the maintenance of the energy output. Since the energy output is primarily, if not entirely, dependent upon the processes of oxidation, a more complete knowledge of the mechanisms involved becomes very essential in order that we may more fully understand the factors which govern metabolism. This author states further that the action of thyroxin is probably that of a catalyst which bears a quantitative relationship to the energy within the tissues and that a linear proportionality exists between the energy production and the supply of thyroxin. The substance seems to function in the tissues; and between the tissues, blood and the thyroid gland there is established a state of equilibrium. He further believes that the chemical reactions in the tissues induced by the administration of thyroxin are not qualitatively different from those fundamental reactions which occur normally in the absence of this hormone. The work of Adler (1920) and Lieb and Hyman (1922) would indicate that the action of the thyroid hormone is on the oxidation processes in the tissues and not on the heat center or nerve endings.

It seems probable that the apparent catalytic activity of thyroxin upon body metabolism may be an indirect one through which it exerts an influence upon the oxidizing systems normally present in the cells. As far as we have been able to find, the only work which deals specifically with this problem is that of Marinesco (1922) who was able to demonstrate histologically a marked diminution of the tissue oxidases in this disease. From a study of various organs from thyroidectomized dogs, in which parathyroid glands had been grafted to prevent the imposing symptoms of tetany,

the absence of oxidases in certain regions of the organs was very striking. In many cases these oxidase-free areas gave positive reaction with Sudan III.

Concerning tissue respiration in relation to thyroidectomy Foster (1927) found the oxygen consumption of tissues from thyroidectomized rats to be markedly diminished. His experimental animals, however, were larger than his normal rats, weighing 155 to 241 grams as compared with 142 to 180 grams for the controls.

SUMMARY

A study of the indophenol oxidase content of heart, kidney, liver and skeletal muscle of normal and thyroidectomized lambs and pups has been made. The influence of temperature on the course and velocity of the reaction is reported and discussed. The optimum temperature lies between 23 and 24°C. An appreciable activity remains at 0°C., while the oxidase is completely inactivated at 55°C. in thirty minutes. Without exception, a marked diminution in indophenol oxidase was found in all the tissues which were studied from the thyroidectomized animals. In lambs, this varied from 24 per cent for heart to 50 per cent for skeletal muscle in twenty minutes, that for kidney and liver being 24 per cent and 20 per cent respectively. Lower values were obtained for pups, in which all tissues seemed to be affected to about the same extent. In these, the decrease ranged from 10 to 20 per cent. These figures compare favorably with the well-known fact that basal metabolism may be reduced 30 to 40 per cent below the normal in cases of hypothyroidism.

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STUDIES ON TISSUE RESPIRATION AND ENDOCRINE FUNCTION

II. The Indophenol Oxidase Content of Animal Tissues after Total Thyroparathyroidectomy in Dogs

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Of the many theories regarding the etiology and pathogenesis of tetany, three stand out quite prominently, viz., 1, calcium deficiency; 2, guanidin intoxication, and 3, alkalosis. The oxygen deficiency theory of Morris (1922) has not been given such wide recognition. This investigator found hyperexcitability of the myoneurone, with accompanying tetany or convulsions, to be very frequently associated with the occurrence of anoxemia under such varied conditions as asphyxia, anemia, alcohol intoxication, lowered body temperature and histamine poisoning. None of the theories which are accepted at the present time will hold for all types of tetany observed clinically. Campbell (1926), in an attempt to harmonize these various forms of tetany by seeking a common cause, found that anoxemia was more often associated with this affection than alkalosis, guanidin poisoning or deficiency of calcium when induced by injections of guanidin, sodium salts, insulin, water, ammonium salts, cyanides, strychnine, inspiration of oxygen under low tensions and parathyroidectomy. He measured the state of oxygen deficiency by determining the tension of this gas in air previously injected under the skin or into the peritoneal cavity. A state of equilibrium is soon established between this encased air and the gases of the circulating fluids of the tissues beneath. This offers a ready means of determining the available oxygen supply in the environment of the adjacent tissue cells. Tetany from parathyroidectomy, cyanide injections or strychnine poisoning, however, was not associated with a decrease in oxygen tension of the tissue fluids. This, moreover, is not necessarily evidence that a state of asphyxia does not exist within the cells, as Campbell (1926) has pointed out. Since cyanide doubtless exerts its influence upon the aerobic oxidation processes of the cells themselves (Fleisch, 1924; Harrison, 1924, 1927), it seems very probable that there may actually be a state of intracellular anoxemia in parathyroid tetany, the immediate cause of which might be demonstrated by special methods.

TABLE 1

Animals designated by the same letter were from the same litter

ANIMAL	AGE	DATE OF THYROIDECTOMY	FIRST INDICATION OF TETANY	DURATION OF TETANY	HIGHEST TEMPERATURE PER RECTUM °F.	ACUTENESS OF SYMPTOMS	RESPIRATION	REMARKS
A ♂ C.	9 months							Killed by overdose of ether. 3/25/27. Collie
A ♂ op.	9 months	3/16/27	48 hours	28 hours	105	Acute	Hyperpnea	Fed 1 pound meat 3/19/27. Died at 1:40 p.m. Collie
A ♀ op.	9 months	3/ 9/27	20 hours	26 hours	102.7	Semi-acute	Moderate	Died in tetany 3/11/27. Collie
B ♀ C.	6 months							Killed by blow over head. 4/7/27. Collie pup
B ♀ op.	6 months	4/4/27	2d day	22 hours	105.5	Acute	Hyperpnea	Fed ½ pound meat, 11 p.m. 4/6/27. Died 4/7/27. Collie pup
C ♂ C.	6 months							Killed by blow over head. 5/22/27. Collie pup
C ♂ op.	6 months	5/17/27	64 hours	54 hours	106	Acute	Hyperpnea	Died 5/22/27. Tissue studied 15 hours later. Collie pup
D ♂ C.	Young adult							Killed by blow over head. 6/9/27. Mongrel
D ♂ op.	Young adult	6/2/27	4th day	2 days	106	Acute	Hyperpnea	Killed by blow over head, 6/9/27. Refused food after 6/7/27. Mongrel
E ♂ C.	Young adult							Killed by blow over head and bleeding from jugular. 2/27/27. Mongrel
F ♀ op.	Adult	3/22/27	48 hours	3 days	104.8	Semi-acute	Hyperpnea	Fed 1 pound meat 3/27/26. Killed the same day. Mongrel
G ♂ op.	Old	3/28/27	2d day	30 hours	102	Semi-acute	Hyperpnea	Died in tetany 3/31/27. White collie

It occurred to us that a possible relationship may exist between the parathyroid glands and the oxidizing enzymes of the animal organism. The embryonal origin of these glands as outgrowths of the epithelium of the third and fourth visceral pouches is at least suggestive that they might be functionally related to the processes of respiration. With these possibilities in mind, a series of experiments is being planned in an attempt

TABLE 2

Showing the relative amounts of oxidation with tissues from normal and thyroparathyroidectomized dogs. The values obtained for the normal animal being taken as unity

ANIMAL		A ♂ C.	A ♂ Op.	A ♀ Op.	B ♀ C.	B ♀ Op.	C ♂ C.	C ♂ Op.	D ♂ C.	D ♂ Op.	E ♂ C.	F ♀ Op.	G ♂ Op.	AVERAGE, CONTROLS	AVERAGE, OPERATED
WEIGHT		18 kgm.	18 kgm.	17 kgm.	12 kgm.	8 kgm.	7.8 kgm.	8 kgm.	22 kgm.	20 kgm.	19 kgm.	22 kgm.	23 kgm.		
AGE		9 months	9 months	9 months	6 months	6 months	6 months	6 months	Young adult	Young adult	Young adult	Adult	Old		
TIME	TISSUE														
minutes															
20	Heart	100	116	108	100	98	100	160	100	104	100	116	76	100	111
40	Heart	100	102	105	100	100	100	170	100	121	100	135	107	100	120
60	Heart	100	107	97	100	99	100	115	100	108	100	127	114	100	110
90	Heart	100	105	99	100	100	100	100	100	100	100	126	116	100	107
120	Heart	100		105	100		100		100		100				
20	Kidney	100	95	97	100	110	100	81	100	127	100	71	79	100	94
40	Kidney	100	105	107	100	108	100	91	100	121	100	85	90	100	101
60	Kidney	100	105	107	100	107	100	93	100	100	100	107	113	100	105
90	Kidney	100	97	104	100	103	100	86	100	98	100	124	112	100	103
120	Kidney	100			100		100	90	100		100				
20	Liver	100	140	146	100	92	100	56	100	137	100	111	75	100	108
40	Liver	100	160	155	100	98	100	50	100	126	100		70	100	110
60	Liver	100	160	147	100	94	100	43	100	82	100	104	62	100	99
90	Liver	100	122	109	100	100	100	58	100	100	100	112	82	100	98
120	Liver	100	104		100	103	100	67	100	100	100	112	97	100	97
20	Muscle	100	164	138	100	113	100	90	100	134	100	81	92	100	116
40	Muscle	100	152	154	100	95	100	76	100	119	100	65	83	100	106
60	Muscle	100	143	128	100	93	100	88	100	112	100	97	89	100	107
90	Muscle	100	108	106	100	100	100	90	100	101	100	110	113	100	104
120	Muscle	100	96	100	100	101	100	104	100	100	100	105	107	100	102

to obtain a better understanding of the facts. In connection with our work on the indophenol oxidase content of tissues from thyroidectomized animals, the study was extended to cases of parathyroid tetany in dogs. The method employed was the same as that described previously (1927).

EXPERIMENTAL. Complete thyroparathyroidectomy was performed on seven dogs. These varied in age from six months to comparative senility

With the exception of the two old animals, they were properly controlled as to sex, age and body weight, in experiments A, B, C and D by dogs of the same litter. A brief history of the protocols is given in table 1. From this table it may be seen that the cases varied somewhat as to the interval before the onset of tetany, acuteness of the attack, highest body temperature and duration of the survival.

Table 2 contains the experimental data from a quantitative study of the ability of tissue from these animals to catalyze the oxidation of a-naphthol and dimethyl-para-phenylene-diamine-hydrochloride into indophenol blue. The amount of oxidation observed for the normal tissue for any given time is taken as unity (100 per cent). Thus an increased or decreased rate of oxidation can be read off directly, e.g., 116 for heart muscle of A ♂ op. in twenty minutes indicates an increase of 16 per cent oxidation above that of A ♂ C. and 95 for kidney of this animal for the same time shows a diminution of 5 per cent. In experiments B, C and D the determinations on any given tissue were run simultaneously for the normal and operated animals. From the table, it can be seen that the figures obtained at the end of two hours are approximately the same for both normal and experimental animals. The reason for this is evident when it is remembered that a definite quantity of the substrate (5 cc.) is used in each test. If sufficient time is allowed, reactions occurring with normal, increased and decreased velocities will ultimately come to an equilibrium at approximately the same level although more time will be required for the slower ones. This equilibrium for normal tissue usually lies at a point below 100 per cent and is reached earlier with heart, while kidney, liver and skeletal muscle follow in descending order.

These facts are best shown in figures 1 and 2 in which curves for all four tissues are given for experiments A, B and D. In these, actual plate determinations, which express the per cent of complete oxidation of the substrate, are plotted against the time. The amount of spontaneous oxidation occurring in any given interval is shown at the bottom of each figure.

Discussion. The varied and seemingly contradictory results obtained and tabulated above appear difficult of interpretation at first, but when analyzed more carefully certain very suggestive correlations are brought out. This is especially the case when the experiments are considered collectively and average values compared. Since, as can be seen from figures 1 and 2, the reactions reach a point of equilibrium between 40 and 90 minutes, and the curves for the experimental tissues tend to approximate those of the normal, it is obvious that any deviation which might exist between them would be most marked between 20 and 40 minutes. The discussion and conclusions which follow will be concerned largely with observations recorded for these time intervals.

The curves in figure 1 are drawn from actual plate determinations of experiment A. From these, it will be observed that the oxidation rate is higher in the experimental tissues than in the normal, none being excepted. This acceleration is particularly marked in liver and skeletal muscle. Similarly figure 2 represents actual plate readings for experiments B and D. In the latter, the curves are higher for each of the organs of the parathyroidectomized animals studied while in B they are higher for kidney and skeletal muscle, lower for liver and practically normal for heart.

An analysis of individual cases from table 2, in which the per cent of oxidation is compared with the normal as unity, shows that an increase in

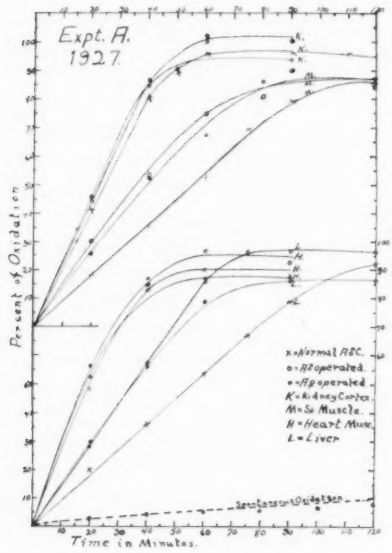

Fig. 1

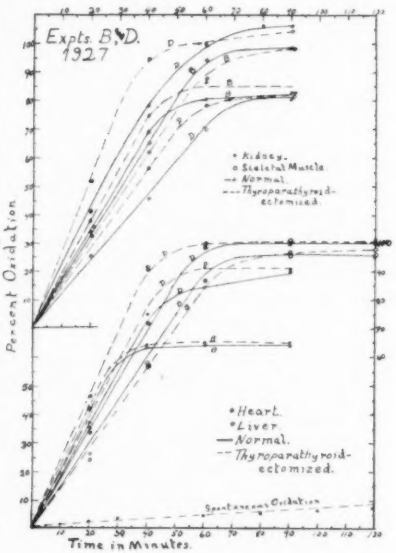

Fig. 2

the velocity of the reaction occurred in all the tissues of A ♂ op. and A ♀ op. except kidney. This was particularly marked for liver and skeletal muscle. On the whole the values for B ♀ op. are quite normal. There is at most a slight increase for kidney and skeletal muscle and a small decrease for liver. In C ♂ op. all values are low except those for heart in which an increase of 60 to 70 per cent is indicated. In D ♂ op. the oxidation power is increased although only to a slight extent, while in F ♀ op. an increase for heart and liver while a decrease for kidney and skeletal muscle is shown. In all the tissues of G ♂ op. a decrease in oxidation is evident.

F ♀ op. and G ♂ op. were older dogs, the latter being quite senile, while the former was middle aged and the mother of the pups used in experiment A. Since the rate of metabolism is diminished in old age, this may explain, in part, the lower oxidation values obtained for these animals. Unfortunately the control for these experiments was a young adult (dog E. C).

When the averages are calculated for any tissue at a given time for the group as a whole, more interesting results are obtained. These figures will be found in the last two columns of table 2. As compared with the normal taken as 100 per cent, an increase in oxidation power of heart, liver and skeletal muscle is indicated, that for kidney being approximately normal. Heart shows an increase of 10 to 20 per cent, liver 8 to 10 per cent and skeletal muscle 6 to 16 per cent.

Since the tissues of the animals in experiment C were not studied until fifteen hours after death, it seems quite probable that the low values obtained might be due to this fact. We have noticed that liver oxidase is particularly reduced when the tissue is allowed to stand after death and in some cases found it to be less than one-half its original value after 24 hours. For comparative purposes, the data obtained from all of the animals has been included. By omitting C ♂ op., F ♀ op. and G ♂ op. from the series, the average increase in oxidation becomes appreciably greater.

Whether this apparent increase in the rate of tissue oxidation is significant cannot be stated definitely at present. At any rate, the evidence does not support the anoxemia theory of Morris (1922). That there is a rise in heat production during the tetanic seizures cannot be denied and it is probable that this is paralleled, to some extent at least, by an augmented oxidation within the cells. This alone would seem to be an inadequate solution for the occurrence of an enhanced tissue oxidation, if one actually exists. It would be unwise to even attempt a tentative explanation until the problem is studied further by other methods. This we hope to do in the near future.

SUMMARY

The power of minced heart, kidney, liver and skeletal muscle from normal and parathyroidectomized dogs to oxidize a-naphthol and dimethyl-para-phenylene-diamine-hydrochloride into indophenol blue was studied in vitro. From a study of the oxidation curves presented, together with a comparison between the relative amounts of substrate oxidized by the tissues from normal and experimental animals, no evidence was obtained in support of the oxygen deficiency theory of Morris (1922). Although the estimated values are not absolutely constant from organ to organ or from animal to animal, an increase in oxidation power is indicated in all tissues, except kidney, when averages of the group taken collectively are

considered. Approximately normal figures were obtained for this tissue. The severity of tetany in the individual experiments was not sufficiently graded to make possible an exact correlation between the amount of increase in oxidation power of the tissues and the acuteness of the tetanic seizures. The probable explanation of these results is withheld until a further study with other methods is completed.

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THE NATURE OF THE FOODSTUFFS OXIDIZED TO PROVIDE ENERGY IN MUSCULAR EXERCISE

II. In The Phlorhizinized Animal

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In our first conception regarding the problem here considered, we made the postulate that in mild exercise of short duration in a normal animal, the respiratory quotient of the excess metabolism of exercise and recovery would be unity. We proposed then to determine whether, as a slowly developing interference with sugar oxidation was produced, the muscles would continue to utilize carbohydrate exclusively as long as the organism retained any capacity to oxidize sugar, and then suddenly shift to fat, or whether the use of fat would quantitatively parallel the extent of the interference, using the D to N ratio as the chief criterion of the latter.

We were soon made aware, however, that our original postulate was incorrect, for in experiments upon normal dogs we found that the animals oxidized approximately the same percentages of carbohydrate and fat in exercise as in rest, the relative amount of each being a function of the preceding diet. We have recently (1928) reported these experiments, concluding that in respect to the foodstuffs used to provide energy, muscles do not appreciably differ, in the normal animal, from the tissues of the organism as a whole, oxidizing carbohydrate preferentially only when this happens to be the most available fuel presented to them.

This changes the aspect of our present problem, in which we are concerned to see if a similar parallelism between the metabolism in exercise and in rest obtains in the phlorhizinized animal, or if in this condition the muscles are more apt to utilize the available carbohydrate supply than are the other tissues. From this point of view alone it is practically immaterial whether the admittedly lessened capacity of the body for oxidizing sugar be due only to a lowering of the renal threshold with a resultant partial or complete lack of carbohydrate to oxidize, or in addition, to an impairment of the oxidative mechanism. Our primary interest was not in the mechanism of phlorhizin diabetes. We have, however, obtained evi-

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dence which points to an actual diminution in the power of the organism to oxidize sugar, even when a hyperglycemia exists, though we have not under the conditions of our experiments observed a complete abolition of this function.

Loebel, Barr, Tolstoi and Himwich (1924) showed that under extreme conditions of phlorhizinization and glycogen depletion the ability to form lactic acid as a result of exercise is unimpaired. Weber, Briggs and Doisy (1925) showed that lactic acid is formed during muscular work in pancreatic diabetes so long as the muscles possess glycogen. They believe that as the store of glycogen is depleted the animal becomes less able not only to form lactic acid but also to perform work. This type of evidence tends to confirm, for the diabetic, the current conception of the initial phase of muscular contraction. It does not of course answer the question of the source of energy in oxidative recovery. Hetzel and Long (1926) have studied the metabolism of human diabetics in muscular exercise. Among other things they find that their subjects, without recent administration of insulin, do not show respiratory quotients of unity even in short mild exercise. However, the R. Q. of the excess metabolism is higher (0.80) than that (0.76) of the resting metabolism, indicating that with a limited supply of carbohydrate available, the muscles are somewhat more apt to use it than are the other tissues.

The experiments which we are reporting in this communication offer evidence that in the phlorhizinized dog the muscles progressively fail to utilize ingested sugar, as do the other tissues, and that under these circumstances the energy for continued muscular contraction is increasingly supplied by fat.

EXPERIMENTAL PROCEDURE. We have observed the metabolism of three female dogs made progressively diabetic with phlorhizin. The animals were made to run on a horizontal treadmill (previously described—1928) at the rate of 1.2 miles per hour for 15 minutes, involving for this period a three- to fourfold increase in the O_2 consumption. The dogs had been trained to run easily and rest quietly before and after running (dogs 7 and 8 had been used in our previous work). We experienced no difficulty in getting them to perform this mild exercise, and they showed no signs of distress after its performance.

The experiments were nearly always carried out shortly after the administration of glucose. In some cases an initial dose of 20 grams of glucose was given, followed by a second dose of 30 or 50 grams four hours later, after which the experiment was begun, the exercise being performed usually at about two hours after the second administration of glucose. In accord with the observations of Wierzuchowski (1926), this method of successive doses was adopted to obviate the possible masking effect of the disappearance of ketosis upon the respiratory quotient. In other experi-

ments the initial dose was omitted. Apart from this glucose, no food was given throughout the experimental period, the earliest experiments being carried out at least two days after the last food.

Phlorhizin (Merck's, recrystallized) was given subcutaneously suspended in oil. Since we wished to observe the developmental course of the diabetes, we employed a dosage of as little as 0.25 gram, and did not necessarily give the drug daily. As the condition developed, the dosage was increased to 1.0 gram, repeated daily. The details of this are given in the protocols.

The respiratory exchange was determined in a closed system of the Benedict-Homans type, previously described in reports from this laboratory. Four alcohol checks were performed in the course of this work to determine the accuracy of the method. In these checks, summarized in table 1, the average R. Q. was 0.671, a mean error of 0.004 (0.6 per cent). The maximum deviation of the R. Q. involved an error of 0.008, or 1.2 per

TABLE 1

Alcohol checks

DATE (1927)	EXPERIMENT NUMBER	CALORIES PER HOUR	R.Q.
June 16	42	13.73	0.666
June 21	43	12.27	0.675
July 5	44	12.14	0.671
July 12	45	12.82	0.673
Average			0.671

Mean R. Q. deviation = 0.004 (0.6 per cent error)
Maximum R. Q. deviation = 0.008 (1.2 per cent error)

cent. Alcohol checks performed in the course of our earlier work proved that alternate starting and stopping of the treadmill, as well as wide variations in the heat production, did not impair the accuracy of the apparatus.

As a routine, upon the administration of sugar the animal was at once introduced into the animal chamber, and after a preliminary period to permit equilibration of conditions within the system, the resting metabolism was obtained during two half-hour periods. This was followed by the 15 minute exercise, and the period was closed after another 15 minutes of rest. The reasons for this procedure have been sufficiently set forth in the previous communication (1928). At the end of another half-hour period, recovery was invariably complete. Additional periods were then run until we were certain that we were obtaining the post-recovery metabolism. In our previous report, we found it advisable to calculate the excess metabolism upon an average of the resting metabolism before and after exercise. In the present work this was quite necessary, in view of the fact that glu-

cose was given shortly before the experiment, and the resting heat production and R. Q. that might have been expected during the time when the animal was actually performing exercise and recovering therefrom, could only be taken as the mean of the resting metabolism before and after these periods.

It was impossible to employ diabetic factors in calculating the metabolism, for the interference with carbohydrate oxidation was in no case complete, and there was no certain way of knowing what percentage of protein was oxidized as sugar in any particular case. On the other hand, calculation of the non-protein metabolism on the ordinary factors would also introduce an error of unknown amount. We have therefore neglected the non-protein R. Q. of the resting periods, and compared the total R. Q. of these periods with the R. Q. of the excess metabolism. The resulting error is slight and does not invalidate our conclusions.

The blood sugar was determined by the Folin-Wu method before and after each experiment. The D to N ratio was established on 24 hour specimens of urine, collection of which was begun usually 48 hours, and never less than 24 hours, after the last previous ingestion of glucose. Urinary sugar was determined by Benedict's method; nitrogen by the macro-Kjeldahl method.

EXPERIMENTAL RESULTS. *a. Experiments on dog 7.* This animal was at the beginning of the experiments a well-nourished but not fat dog weighing 8.9 kilos, the weight dropping finally to 7.5 kilos. The first experiment (no. 14) was an exception to the rule that the animals were not given a daily ration, for in this case a daily diet of 200 grams lean meat and 40 grams lard was given for three days before the experiment was performed. One-fourth gram of phlorhizin was given on each of the two days preceding the experiment. The D to N ratio of the 24 hour period ending on the morning of the experiment was 3.00:1. Fifty grams of glucose were given by stomach tube 2 hours and 4 minutes before the exercise was begun. The average resting respiratory quotient was 0.83; that of the periods of exercise and recovery 0.82. From this the R. Q. of the excess metabolism was calculated as 0.79 (table 2).

The animal was then allowed to recover and on June 21 starvation was begun and continued thereafter except when glucose was administered. Experiment 15, on June 23, showed the reaction of the starving animal to 50 grams of glucose. The resting R. Q.'s before and after exercise were respectively 1.015 and 1.027, while that of the exercise and recovery metabolism was 0.97. The excess metabolism therefore had a respiratory quotient of 0.91. The blood sugar at the end of the experiment was 0.127 per cent. The phenomenon that we had previously observed (1928), namely, an apparent cessation of fat production from carbohydrate when the demand for fuel was increased, was to be seen in this experiment.

TABLE 2

Résumé of experiments on dog 7

EXPERIMENT NUMBER	DATE	BASAL (BEFORE EXERCISE)		EXERCISE AND RECOVERY		BASAL (AFTER EXERCISE)		EXCESS		REMARKS	
		O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q.		
	1927	*gm. per hr.*		*gm. per hr.*		*gm. per hr.*		*gm.*			
14	June 15	4.90	0.825	7.46	0.816	4.78	0.840	2.62	0.786	Diet*: Meat 200 gm., lard 40 gm. Blood sugar at 9:30 a.m. = 0.065 per cent Exercise: 12:11 to 12:26 p.m. Blood sugar at 2 p.m. = 0.165 per cent D : N (June 14–15) = 3.00:1	Phlorhizin, 0.5 gm. during 2 days 50 gm. glucose at 9:30 a.m.
15†	June 23	4.68	1.015	6.69	0.970	4.42	1.027	2.14	0.905	Diet: Starvation since June 21 Blood sugar at 10:45 a.m. = 0.087 per cent Exercise: 1:05 to 1:20 p.m. Blood sugar at 2:55 p.m. = 0.127 per cent	50 gm. glucose at 10:55 a.m.
16	June 25	5.42	0.730	7.33	0.743	Not obtained		1.91	0.780	Diet: Starvation since June 21 Exercise: 9:38 to 9:53 a.m.	Phlorhizin, 0.75 gm. during 2 days
17	June 25	5.64	0.823	7.20	0.840	5.58	0.863	1.59	0.835	Diet: Starvation since June 21 Blood sugar at 11:05 a.m. = 0.067 per cent Exercise: 1:11 to 1:26 p.m. Blood sugar at 3 p.m. = 0.161 per cent D : N (June 24–25) = 3.42 : 1	Phlorhizin, 0.75 gm. during 2 days 50 gm. glucose at 11:08 a.m.
18	June 29	5.37	0.760							Diet: Starvation since June 21 No exercise. Basal from 9:17 to 11:17	Phlorhizin, 4.25 gm. during 6 days 20 gm. glucose at 8:15 a.m.
19	June 29	5.20	0.797	6.95	0.813	5.18	0.842	1.76	0.792	Diet: Starvation since June 21 Blood sugar at 11:50 a.m. = 0.143 per cent Exercise: 2:43 to 2:58 p.m. Blood sugar at 4:25 p.m. = 0.132 per cent D : N (June 28–29) = 3.56 : 1	Phlorhizin, 4.25 gm. during 6 days 20 gm. glucose at 8:15 a.m. 50 gm. glucose at 12:10 p.m.

* "Diet" refers to maintenance diet which, when administered, is given at 4:30 p.m.

† Animal allowed to recover from phlorhizinization between experiments 14 and 15.

On June 23 the animal was given 0.25 gram of phlorhizin and on June 24, 0.5 gram. On June 25 the animal was exercised without being given glucose. The resting R. Q. was 0.73; the total R. Q. of exercise and recovery, 0.74; the excess R. Q., 0.78. Then 50 grams of glucose were given

and the metabolism again determined. The resting R. Q. was now 0.84 and the excess R. Q., 0.83. The D:N of the day before was 3.42 :1. The blood sugar before glucose was given was 0.067 per cent; five hours later, after the completion of the experiment, it was 0.161 per cent. There was certainly no lack of circulating blood sugar, and on the basis of the results obtained by Nash and Benedict (1923) upon injecting glucose into phlorhizinized animals, it is clear that at the time of the exercise there must have been a definite hyperglycemia.

Administration of phlorhizin was continued, and by June 29 the dog had received 4.25 grams, and had a D:N of 3.56:1. Twenty grams of glucose were given on this day and the resting metabolism determined, the R. Q. averaging 0.76 over a period of two hours. Then 50 grams of glucose were administered, following which the resting quotient was 0.82 and the excess quotient 0.79. The blood sugar at the end of the experiment, four hours after glucose and 1½ hour after the exercise, was 0.132 per cent.

Thereafter 1 gram of phlorhizin was administered daily, but no further experiments could be performed, as the animal would not retain glucose.

The experiments on this animal showed in rest and exercise an equal diminution of the capacity to oxidize sugar, in spite of the presence of a blood sugar content at least as great as that seen after glucose ingestion when the animal was not under the influence of phlorhizin.

b. Experiments on dog 8. The weight of this dog, a fat spaniel, dropped from 9.2 to 7.6 kilos during the experiments. Experiment 6, two days after the last food, showed a resting R. Q. of 0.80 and an excess R. Q. of 0.84 (table 3). After 50 grams of glucose on the same day the resting quotient was 0.94 and the quotient of the excess metabolism 1.01, representing the normal reaction to the ingestion of a fairly large amount of sugar. The blood sugar at the end of the experiment (no. 7), four hours after the glucose administration, was 0.078.

Three days later, after 0.25 gram of phlorhizin on the previous day, a starvation metabolism, that is, one not preceded by glucose ingestion, showed a resting respiratory quotient of 0.71 and a total quotient of exercise and recovery of 0.73, indicating an excess quotient of 0.79. After 50 grams of glucose the resting R. Q. was 0.82 and the excess quotient 0.79. The blood sugar jumped from the starvation level of 0.069 per cent to 0.120 per cent at the end of the experiment. Thereafter, on the continued administration of phlorhizin, in two sets of experiments (table 3), the respiratory quotients both at rest and in exercise were not elevated to any extent by the administration of 50 grams of glucose, all of the quotients varying only within the limits of 0.71 and 0.74.

However, a critical experiment on July 2 (expt. 14) showed that the oxidation of glucose had apparently been masked by its effect on the CO_2 carrying power of the blood, for when the administration of 20 grams of glucose

TABLE 3

Résumé of experiments on dog 8

EXPERIMENT NUMBER	DATE	BASAL (BEFORE EXERCISE)		EXERCISE AND RECOVERY		BASAL (AFTER EXERCISE)		EXCESS		REMARKS	
		O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q		
	1927	*gm. per hr.*		*gm. per hr.*		*gm. per hr.*		*gm.*			
6	June 17	3.90	0.793	6.42	0.801	3.86	0.773	2.54	0.838	Diet*: Starvation since June 15 Exercise: 10:34 to 10:49 a.m.	
7	June 17	4.56	0.957	7.17	0.966	5.22	0.933	2.28	1.012	Diet: Starvation since June 15 Exercise: 2:51 to 3:06 p.m. Blood sugar at 4:58 p.m. = 0.078 per cent	50 gm. glucose at 12:50 p.m.
8	June 20	4.94	0.706	7.34	0.725	Not obtained		2.40	0.785	Diet: Starvation since June 15 Exercise: 11:23 to 11:38 a.m.	Phlorhizin, 0.25 gm. during 1 day
9	June 20	4.92	0.803	8.33	0.812	5.92†	0.766	3.41	0.790	Diet: Starvation since June 15 Blood sugar at 1:28 p.m. = 0.069 per cent Exercise: 3:26 to 3:41 p.m. Blood sugar at 5:22 p.m. = 0.120 per cent	Phlorhizin, 0.25 gm. during 1 day 50 gm. glucose at 1:25 p.m.
10	June 24	4.82	0.729	7.15	0.733	5.18	0.730	2.15	0.743	Diet: Starvation since June 15 Exercise: 9:41 to 9:56 a.m.	Phlorhizin, 0.75 gm. during 5 days
11	June 24	5.30	0.754	7.16	0.732	4.96	0.718	2.02	0.723	Diet: Starvation since June 15 Blood sugar at 11:46 a.m. = 0.045 per cent Exercise: 1:59 to 2:14 p.m. Blood sugar at 4:28 p.m. = 0.143 per cent D : N (June 23–24) = 3.10 : 1	Phlorhizin, 0.75 gm. during 5 days 50 gm. glucose at 11:58 a.m.
12	June 27	5.34	0.714	7.16	0.713	5.86†	0.717	1.82	0.721	Diet: Starvation since June 15 Exercise: 10:39 to 10:54 a.m.	Phlorhizin, 2.75 gm. during 8 days
13	June 27	5.28	0.713	8.59†	0.711	5.16	0.708	2.61	0.711	Diet: Starvation since June 15 Blood sugar at 12:32 p.m. = 0.048 per cent Exercise: 2:36 to 2:51 p.m. Blood sugar at 4:25 p.m. = 0.156 per cent D : N (June 26–27) = 3.21 : 1	Phlorhizin, 2.75 gm. during 8 days Glucose 50 gm. at 12:35 p.m.
14	July 2	4.75	0.767	7.02	0.791	4.88	0.793	2.20	0.814	Diet: Starvation since June 15 Blood sugar at 8:55 a.m. = 0.056 per cent Exercise: 11:14 to 11:29 a.m. Blood sugar at 1 p.m. = 0.136 per cent D : N (July 1–2) = 3.08 : 1	Phlorhizin, 6.75 gm. during 13 days 20 gm. glucose at 5 a.m. 50 gm. glucose at 9 a.m.

* "Diet" refers to maintenance diet which, when administered, is given at 4:30 p.m.

† Restless.

was followed by 50 grams four hours later, the ensuing R. Q. at rest was 0.78 while the total R. Q. of exercise and recovery was 0.79, from which the

TABLE 4

Résumé of experiments on dog 9

EXPERIMENT NUMBER	DATE	BASAL (BEFORE EXERCISE		EXERCISE AND RECOVERY		BASAL (AFTER EXERCISE)		EXCESS		REMARKS	
		O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q.		
	1927	*gm. per hr.*		*gm. per hr.*		*gm. per hr.*		*gm.*			
1	July 8	4.50	0.727							Diet*: Starvation since July 5 No exercise. Basal 8:09 to 9:09 a.m.	
2	July 8	4.80	0.907	8.01	0.896	5.32	0.946	2.95	0.874	Diet: Starvation since July 5 Blood sugar at 9:22 a.m. = 0.072 per cent Exercise: 3:23 to 3:38 p.m. Blood sugar at 5:30 p.m. = 0.083	20 gm. glucose at 9:20 a.m. 30 gm. glucose at 1:20 p.m.
3	July 9	4.77	0.953	7.21	0.945	5.14	0.937	2.25	0.947	Diet: Starvation since July 5 Blood sugar at 11:55 a.m. = 0.060 per cent Exercise: 2:07 to 2:22 p.m. Blood sugar at 4:00 p.m. = 0.057 per cent	Phlorhizin, 0.25 gm. during 1 day 20 gm. glucose at 8:05 a.m. 30 gm. glucose at 12:05 p.m.
4	July 11	4.77	0.874	7.50	0.824	4.97	0.878	2.43	0.728	Diet: Starvation since July 5 Blood sugar at 12:10 p.m. = 0.075 per cent Exercise: 2:20 to 2:35 p.m. Blood sugar at 5:00 p.m. = 0.071 per cent D : N (July 10–11) = 3.38 : 1.	Phlorhizin, 1.0 gm. during 3 days 20 gm. glucose at 8:15 a.m. 30 gm. glucose at 12:15 p.m.
5	July 13	4.63	0.823	6.93	0.816	4.57	0.891	2.33	0.734	Diet: Starvation since July 5 Blood sugar at 12:30 p.m. = 0.065 per cent Exercise: 2:53 to 3:08 p.m. Blood sugar at 5:00 p.m. = 0.110 per cent D : N (July 12–13) = 3.73 : 1.	Phlorhizin, 2.0 gm. during 5 days 20 gm. glucose at 8:30 a.m. 30 gm. glucose at 12:40 p.m.

* "Diet" refers to maintenance diet which, when administered, is given at 4:30 p.m.

excess R. Q. was calculated as 0.81. Thus the giving of 6.75 grams of phlorhizin over a period of 13 days had been unable entirely to abolish the oxidation of carbohydrate, to which about 30 per cent of the energy ex-

change was due during both rest and exercise plus recovery. On this day the blood sugar rose from the fasting level or 0.056 per cent to 0.136 per cent four hours after 50 gm. of glucose had been ingested.

c. Experiments on dog 9. This animal was a small, well nourished, but not fat poodle whose weight dropped from 6.9 kilos to 6.5 kilos during the experiments. Glucose was given in successive doses, an initial dose of 20 grams and four hours later another one of 30 grams, following which the experiment was performed. In a basal metabolism, $2\frac{1}{2}$ days after the last food, the R. Q. was 0.73. After the ingestion of glucose the excess quotient was 0.87, somewhat lower than the usual normal response (table 4). This day 0.25 gram of phlorhizin was given, and on the following day the drug had apparently not affected the normal capacity to oxidize sugar, for after glucose the resting respiratory quotient and that of the excess metabolism of exercise were both 0.95. The blood sugar was low both before and after the experiment, being 0.060 per cent and 0.057 per cent respectively.

Two days later (expt. 3), after 1.0 gram of phlorhizin over a period of three days, the resting R. Q. following glucose ingestion was 0.88. The excess R. Q., however, was 0.73. The blood sugar before and after the experiment was 0.075 per cent and 0.071 per cent, respectively. On repeating this experiment two days later, when the dog had received a total of 2.0 grams of phlorhizin, almost identical results were obtained. The resting respiratory quotient was 0.869; that of the excess metabolism was 0.73. The blood sugar before the experiment was 0.065 per cent; five hours after the ingestion of the last dose of glucose it was 0.110 per cent, a higher level than we had before observed in this dog.

It appears therefore that in this animal the capacity of the muscles to oxidize glucose was diminished to a considerably greater degree than that of the organism as a whole.

The dog seemed to be peculiarly susceptible to phlorhizin, for after the administration of another gram of the drug convulsions occurred, and the experiments were discontinued.

In this animal the maximum D:N of 3.73:1 was not reached until the day of the final experiment. Two days previously, when the metabolism indicated practically the same percentage oxidation of carbohydrate, the D:N was 3.38:1. In dog 7, a D:N of 3.42:1 was obtained after four days of starvation and two days of phlorhizinization, during which 0.75 gram of the drug was given. Another D to N ratio, four days later, after 4.25 grams of phlorhizin had been given, was 3.56:1, or practically the same as before. In both cases the starvation R. Q. indicated the oxidation of some carbohydrate, and the ingestion of sugar showed further that the capacity to burn sugar had not been entirely lost. In dog 8, the fattest animal of the three, the maximum D:N was lower than in that of the other dogs (3.21:1). It did not appreciably change during the course of the experiments.

Discussion. Deuel, Wilson and Milhorat (1927) are of the opinion that phlorhizin diabetes is of renal origin, and that no impairment of the carbohydrate oxidizing mechanism exists in this condition. To explain the almost complete recovery of ingested or injected glucose observed by many workers, they postulate in addition to an immediate excretion of sugar, a temporary storage of glycogen, which is later excreted as the blood sugar falls. One may assume that in the presence of a hyperglycemia there are three possible pathways in muscle for the disappearance of sugar: 1, conversion to glycogen; 2, conversion to lactic acid; 3, oxidation. It has been proved by Loebel, Barr, Tolstoi and Himwich (1924) that the second pathway is not interfered with in complete phlorhizinization. On the other hand, the oxidation of carbohydrate is not impaired in conditions of glycogen depletion in the normal animal, such as starvation. If then a failure of sugar oxidation should occur in the presence of a hyperglycemia, one must suppose either that glycogenesis becomes the pathway of choice even in the presence of a need for oxidation and a normal oxidative mechanism, or that there is in fact an impairment of the mechanism of oxidation.

In either case, it suffices for our argument concerning the intermediary metabolism if we are able to assume that under the conditions of these experiments the organism is less able, for whatever reason, to oxidize carbohydrate than is the normal animal. It appears from these experiments that an impairment in this sense existed. The basal metabolism under conditions of phlorhizinization and starvation was greater than that of starvation alone. This was associated with a large rise in protein metabolism. In dog 7 the urinary nitrogen increased from 0.095 gram per hour before phlorhizinization to a maximum of 0.240 gram per hour; in dog 8, from 0.090 gram to 0.263 gram; in dog 9, from 0.083 gram to 0.443 gram. Moreover, the resting respiratory quotients after the ingestion of glucose rose much less than in the normal animal even though there was, if anything, a greater rise in the blood sugar. However, the evidence indicates that at no time was the diabetes complete. For one thing, in no case did the R. Q. drop to the level of 0.69. Also, when the masking of the R. Q. by disappearance of ketosis was ruled out, the ingestion of sugar did not fail to produce some rise in the quotient, even when, as in dog 8, 6.75 grams of phlorhizin had been administered over a period of 13 days. This is in harmony with the observations of Wierzuchowski (1926) and of Deuel, Wilson and Milhorat (1927).

In two of the three dogs the respiratory quotients of the excess metabolism of exercise and recovery corresponded very closely to those of the resting metabolism. This does not differ markedly from the findings of Hetzel and Long (1926), in whose experiments on diabetics to whom insulin had not recently been given the excess quotient was 0.80 as compared with 0.76 in the resting state. We are unable, however, to agree with their in-

terpretation of the results. These they take to indicate that in the diabetic the conversion of fat to carbohydrate, so that the latter may be oxidized to provide the energy of recovery from exercise, occurs after exercise of shorter duration than would require such a conversion in the normal individual. If this be true, one would expect to see increasingly lower quotients of the excess metabolism as the exercise is prolonged. An analysis of the figures which make up their average, however, does not reveal this. On exercise of 0.5 minute, the resting and excess R. Q.'s were respectively 0.77 and 0.79; of 1.0 minute, 0.77 and 0.85; of 3 minutes, 0.73 and 0.82; of 5 minutes, 0.74 and 0.75; and of 8 minutes, 0.76 and 0.83. In single individuals also the correlation with the theory is not evident.

In the dogs of our series the evidence indicates that as the diabetes developed increasing proportions of fat were oxidized to provide the energy both for the resting metabolism and that of exercise. In dog 9 either the capacity of the muscles to oxidize carbohydrate, or the amount of carbohydrate in the muscle tissue available for oxidation, diminished even more rapidly than in the case of the other tissues, causing the excess R. Q. to approach the theoretical quotient for fat more rapidly than did the resting R. Q. We are obliged to conclude therefore that as in the case of the normal animal, the muscles of phlorhizinized animals show no greater tendency to oxidize carbohydrate than do the tissues of the whole organism.

SUMMARY

1. Experiments are reported in which the excess metabolism of exercise and recovery has been studied in three dogs that were caused to run on a horizontal treadmill during the development of phlorhizin diabetes. The experiments were performed following the administration of glucose.

2. In two of the animals there was a close correspondence between the respiratory quotients in rest and in exercise. In the third animal the respiratory quotient of the excess metabolism was lower, after phlorhizinization, than that of the resting metabolism, and approached the theoretical value for fat.

3. The results indicate that in the phlorhizinized animal, even though a hyperglycemia exist, there is a diminution in the oxidation of carbohydrate by the organism as a whole, the muscles sharing in this to at least an equal degree. Under these circumstances the fuel requirement in both rest and exercise is provided by the oxidation of fat to a greater extent than in the normal animal.

4. Under the conditions of the experiments the ability to oxidize sugar was not entirely lost, although D to N ratios approximating 3.65:1 were obtained.

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A STUDY OF THE EFFECT OF YEAST UPON METABOLISM

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Smith (1), Salomon (2), Funk, Lyle and McCaskey (3), Murlin and Mattill (4) and others have investigated the effect on the metabolism of human subjects when yeast was added to a diet in such quantities as are commonly recommended for therapeutic purposes. The results reported have shown considerable variation. Such discrepancies are not surprising when we consider the difficulty of establishing and maintaining anything like uniform conditions of eating, exercise, sleep and nervous control in human beings. A satisfactory metabolism study must include many subjects or extend over a long period of time.

For this reason, in spite of the careful work reported by others, further dietary studies with and without yeast seemed important both for confirmation and additional information. We wished particularly to extend the experimental periods so that we might obtain several sets of data for each interval.

Plan of experiments. The study was carried through three separate dietary periods. In the first period six persons, three men and three women, continued on a weighed diet for twenty-seven days, two days of which were used for practice in weighing the food and collecting samples. The subjects were all normal laboratory workers. Breakfasts and dinners were prepared and served in the home of one of the subjects. Luncheon was eaten in the laboratory. All food was weighed and the liquids were measured. Breakfast consisted of orange juice, cereal (shredded wheat, grape nuts or puffed wheat), toast, butter, sugar, cream, coffee. All but one of the subjects took coffee. Lunch consisted of bread, Uneeda biscuits, Kraft cheese, lettuce, jelly, bananas and milk. Dinner consisted of baked potato, vegetable (carrots, beets or green beans), bread, butter, eggs, ice-cream, cream and coffee. Only three took coffee in the evening.

Each subject chose from the above list of foods the kind and amount of each which he considered would be most satisfactory from the standpoint of palatability and quantity. It was a low purine diet and the attempt was made to keep it low in protein, although the primary consideration was the choice of a diet that would not grow too tiresome when repeated day

after day. With one exception the daily protein intake was under 60 grams. The subjects agreed that monotony of the diet did not become a factor of sufficient importance to influence results. However, the nervous strain of coming to meals, weighing the food and keeping up a full program of laboratory work did, we feel, influence certain phases of the work.

The first dietary period of twenty-five days was divided as follows: three intervals of three days on control diet without yeast, two intervals of four days with three cakes of yeast daily, one at each meal, followed by two intervals of four days without yeast. This gave three sets of determinations for the control diet without yeast, two for the yeast period and two for the after-period.

The feces were marked with carmine for each period. The total weight of the feces together with the moisture, total nitrogen and total phosphorus content were determined for each period. The urines were also collected for each period, preserved with toluol, stored in the cold and the composite samples then analyzed.

Samples of blood were drawn on the last day of each interval between 11:00 and 11:30 a.m. The non-protein nitrogen, urea nitrogen, uric acid, creatinine and total phosphorus were determined.

Other investigators have suggested that the effect of yeast on metabolism is a gradual one and can be observed only after an extended period of administration. For this reason we planned a second experiment on which the food intake was limited for three weeks to a low protein, low purine diet as nearly like the control diet as was convenient. This preliminary period was followed by eleven days during which the food was weighed. The same low protein, low purine intake with three cakes of yeast daily was maintained without weighing for twenty-four days. After this the weighed control diet was again resumed for eleven days continuing the three cakes of yeast daily.

In the first set of observations the total nitrogen values did not reach a constant level in every case until the third interval of three days. For this reason in the second experiment the collection of samples was not begun until the total urinary nitrogen had reached a fairly constant level. Five days on the weighed diet were allowed in each case for this. Total nitrogen values fluctuated very little, the greater variations occurring with the higher protein intakes.

Only four of the subjects participated in the second experiment. Collections of urines were made for three intervals of two days during both the yeast and non-yeast period. Feces were collected for the entire six days and the mixed sample analyzed. Blood was drawn for analysis each second day.

Since we observed only slight or no changes in the constituents of the blood and urine with the low purine, low protein diets we felt the study, to

be complete, should include a period of considerably higher protein and purine intake. Two members of the group who had shown a relatively high uric acid excretion acted as subjects for these observations. In one case the protein intake was increased from 41 to 74 grams and in the other case from 53 to 87. Meat was included this time to increase the uric acid output.

TABLE 1

Nitrogen of urine and feces

First experiment

	CONTROL PERIOD AVERAGE OF 3 ANALYSES			YEAST PERIOD AVERAGE OF 2 ANALYSES			AFTER PERIOD AVERAGE OF 2 ANALYSES		
	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
E. S.	9.68	1.79	11.47	9.53	1.54	11.07	9.35	1.51	10.86
E. K.	7.39	0.92	8.31	7.52	1.16	8.68	6.83	1.06	7.89
Z. L.	8.23	0.74	8.97	8.96	0.38	9.34	8.51	0.76	9.27
H. W.	7.94	2.62	10.56	8.56	1.91	10.47	7.81	1.79	9.60
O. H.	11.92	0.99	12.91	11.53	1.73	13.26	11.09	1.40	12.49
G. H.	9.02	1.39	10.41	7.44	1.40	8.84	8.17	1.05	9.22
Average	9.03	1.41	10.44	8.95	1.28	10.23	8.6	1.26	9.86

Second experiment

	CONTROL PERIOD AVERAGE OF 3 ANALYSES			YEAST PERIOD AVERAGE OF 3 ANALYSES		
	Urine	Feces	Total	Urine	Feces	Total
E. S.	8.86	1.13	9.99	8.82	1.26	10.08
E. K.	7.32	0.98	8.30	7.53	1.06	8.59
Z. L.	8.37	0.86	9.23	8.52	0.96	9.48
O. H.	11.18	1.10	12.28	11.40	1.29	12.69
Average	8.93	1.02	9.95	9.03	1.14	10.17

Third experiment

		YEAST PERIOD AVERAGE OF 3 ANALYSES			AFTER PERIOD AVERAGE OF 3 ANALYSES		
		Urine	Feces	Total	Urine	Feces	Total
E. S.		13.21	1.65	14.86	13.17	1.58	14.75
E. K.		13.06	0.93	13.99	12.14	1.13	13.27
Average		13.13	1.29	14.43	12.65	1.36	14.01

For three weeks this high protein intake was maintained approximately without weighing the food. Three cakes of yeast were ingested daily. Seven preliminary days on the weighed diet then followed before collections were started. Three periods of two days each with yeast were followed by three periods of two days each without yeast.

RESULTS. In tabulating the results averages were made of the two or three analyses for each experimental period.

Total urinary nitrogen. Nitrogen was determned by the macro-Kjeldahl method. Three of the six subjects, E. K., Z. L. and H. W., showed an increase in the urinary nitrogen during the yeast period followed by a drop in the after period (table 1). In the two subjects, E. S. and O. H., there was a continuous decrease during all three periods of the experiment, which suggests that these subjects had previously been on a very high protein intake and were gradually being adjusted to the low protein level. All five of these individuals excreted more urinary nitrogen in the yeast period than in the after period. One subject, G. H., showed a decrease in urinary nitrogen during the yeast period and a rise in the after period. The only explanation that suggests itself for this variation is that it may have been due to overlapping of collections since there was no increase in *fecal* nitrogen during the yeast period, and since the average of the control and yeast periods was of the same order as the value for the after period.

In the second experiment where yeast was ingested continuously for four weeks, the urinary nitrogen was greater during the yeast period in three of the four subjects and was the same in the fourth subject, E. S. However, even in this case, the sum of the urinary and fecal nitrogen showed a net increase of 90 mgm. during the yeast period. The additional nitrogen ingested in the yeast was about 99 mgm.

In the third experiment on the high protein where again yeast had been ingested for several weeks both subjects showed a higher urinary nitrogen during the yeast period than during the after period.

An effort was made during experiments II and III to establish a more constant level of nitrogen excretion before beginning collections of urine and feces. This probably explains the more consistent results obtained.

Since some of the subjects showed small fluctuations in weight during the experimental periods the milligrams of urinary and total nitrogen per kilogram of body weight were computed. On this basis the results are only slightly changed and do not alter in any way the general conclusions.

Fecal nitrogen. In the first set of observations there was the same irregularity in fecal nitrogen when comparison is made between the control and the yeast periods as was found in urinary nitrogen (table 1). There was, however, in every case but one, Z. L., more nitrogen excreted in the feces during the yeast period than during the after period. Subject Z. L. was extremely constipated throughout the experiment. She stated that the condition was somewhat relieved during the after period but at no time was entirely comfortable until after the close of the experiment when a diarrhea ensued.

The *sum* of the urinary and fecal nitrogen was higher in the yeast period than in the control period for three subjects and higher than the after pe-

riod for five subjects. One subject, G. H., was erratic in nitrogen excretion.

In the second experiment every one of the four subjects showed a higher fecal nitrogen during the yeast period when compared with the control period. In one case, E. S., the sum of the increased urinary and fecal nitrogen was just about equal to the extra ingested nitrogen in the yeast. In the other three cases the increased urinary and fecal nitrogen was greater than the yeast nitrogen ingested. Other work which is in progress confirms this elimination of nitrogen in excess of that ingested in the yeast. Most of this increase was found in the feces together with the increased phosphorus elimination and indicates poor utilization of yeast nitrogen and phosphorus. In this we do not confirm the findings of Murlin and Mattill (4). Part of this increased fecal nitrogen during the yeast period may be due to greater secretory activity rather than to actual loss of food nitrogen.

During the long period on yeast in the third experiment, both of the subjects felt well. In one subject, E. S., the fecal nitrogen was higher during the yeast period than during the after period. The opposite is true for the other subject, E. K. A rather marked diarrhea in the after period complicated the results in this subject. The diarrhea was of such intensity that the moisture content increased from 73 per cent to 84 per cent. But in spite of the greater *fecal* nitrogen of the after period, the total urinary and fecal nitrogen of the yeast period was 0.72 gram greater than for the after period.

Subject E. S. remained apparently normal after taking carmine. E. K. seemed sensitive to all methods of marking feces and especially so in this period. This may be the explanation of the unusual findings in the case of E. K.

Urea, ammonia, creatinine nitrogen, total urinary acidity and formol titration did not in any case show variations which can be correlated with the ingestion of yeast. The non-protein nitrogen, urea nitrogen and creatinine of the blood were at all times within normal values. For the sake of brevity these data are omitted.

Uric acid. The Koch modification of the Folin direct colorimetric method was used for the determination of uric acid because it eliminates the turbidity which so frequently causes difficulty when the original directions are used. The most notable observation during the first twenty-five day experiment with respect to the uric acid nitrogen excretion was the gradual decrease during the entire period (table 2). If the daily average is made for the nine day control period, the eight day yeast period and the eight day after period, there was a steady decrease in every case but two, Z. L. and E. S. There was a very slight increase, 0.01 gram, *during* the yeast period for Z. L. and a very slight increase, 0.004 gram, *after* the yeast period for E. S. The increase of 0.01 gram might possibly be ascribed to

the yeast since for this individual the original uric acid value was low. In the second case the increase, 0.004 gram, was so slight as to be well within the limits of experimental error for uric acid determinations.

Of significance also is the fact that while the daily values for the first nine day control period showed a range of 0.143 gram to 0.084 gram, the uric acid nitrogen for the same individuals during the last eight day period

TABLE 2

Uric acid nitrogen

First experiment

	CONTROL PERIOD AVERAGE OF 3 ANALYSES	YEAST PERIOD AVERAGE OF 2 ANALYSES	AFTER PERIOD AVERAGE OF 2 ANALYSES
E. S.	0.143	0.107	0.111
E. K.	0.130	0.128	0.108
Z. L.	0.104	0.114	0.092
H. W.	0.084	0.08	0.076
O. H.	0.122	0.114	0.098
G. H.	0.132	0.105	0.099
Average	0.119	0.108	0.097

Second experiment

	CONTROL PERIOD AVERAGE OF 3 ANALYSES	YEAST PERIOD AVERAGE OF 3 ANALYSES	
E. S.	0.144	0.158	
E. K.	0.137	0.146	
Z. L.	0.136	0.115	
O. H.	0.133	0.137	
Average	0.137	0.139	

Third experiment

		YEAST PERIOD AVERAGE OF 3 ANALYSES	AFTER PERIOD AVERAGE OF 3 ANALYSES
E. S.		0.210	0.177
E. K.		0.204	0.161
Average		0.207	0.169

are 0.111 and 0.076 gram. The other members of the group gave uric acid nitrogen values during the after period of 0.092, 0.098, 0.099 and 0.108. That is, all individuals in the group tended toward a constant level for daily uric acid nitrogen excretion. Only H. W. who drank no coffee gave values below 0.09 gram throughout the experiment.

In the second experiment the subjects were seven days on the weighed diet before collections were made. In spite of this there was a considerable

decrease in daily uric acid nitrogen output after the first period. Except in one instance the daily average uric acid nitrogen for the six day no-yeast period was of the same order as for the same individual in the nine day no-yeast period of the first experiment. In the case of the one exception that value was higher. At no time did these values fall as low as those at the end of twenty-seven days on the diet. Apparently a much longer time is required for the uric acid nitrogen to reach a constant level than is the case for total nitrogen.

The average daily uric acid nitrogen output for the yeast period of the second experiment was, in every case but one, slightly higher than for the corresponding no-yeast period. In the one exception there was a lower value for the yeast period. This one subject who had an increased uric acid nitrogen output for the first yeast period and a decreased uric acid nitrogen for the second yeast period was exceedingly constipated during the first experiment due to the interruption of regular habits and the nervous strain of catching an early train before breakfast. During the second experiment, on the other hand, when she ate breakfast at home she observed an increasing tendency toward diarrhea during the entire five weeks she was taking yeast. The result of this was a more rapid rate of elimination.

The slightly higher uric acid nitrogen output of the other three subjects is not surprising. Collections were begun in both the control and yeast periods after five days on the weighed diet. In no case did the uric acid nitrogen values fall so low as in the first experiment where the yeast was ingested from the tenth to seventeenth day on the weighed diet. Our own observations in a third experiment and the results reported by other workers indicate that the addition of three cakes of yeast daily does cause an increase in the urinary uric acid except when the output has already reached a minimum value on a low purine diet.

For the third experiment two of the subjects increased their protein intake. The three cakes of yeast daily were continued for five weeks and an effort made to eat a *high* protein diet. Then the diet was weighed for thirteen days, one subject increasing the protein from 41 grams (the protein intake of the first and second experiments) to 74 grams and the other from 53 to 87.6 grams. Meat was substituted for eggs in the dinner. The daily uric acid nitrogen was distinctly higher during this period of high protein plus yeast ingestion and fell noticeably when the yeast was discontinued. This agrees with the observations of Smith (1) on the one hand, who found that subjects on a purine-free diet showed no increased urinary uric acid when yeast was added, and with the observations of Murlin and Mattill (4), on the other hand, who found that on a high protein, meat containing diet three cakes of yeast daily did cause an increase in uric acid excretion.

However, we did not observe the lag in uric acid excretion reported by Mattill and Murlin since in our subjects the increased elimination occurred very promptly.

The fear is often expressed that the indiscriminate administration of yeast may increase the blood uric acid and that this is especially to be looked for after the long continued use of yeast. For this reason blood uric acid was run by the Koch modification of the direct method. This method gives for normal individuals values of 4 to 5 mgm. per 100 cc. of blood. The administration of yeast either for the short or the long period for the high or low protein diet caused no variations in blood uric acid greater than are found for normal individuals with any of the uric acid methods at present in use.

Phenols. The formation of phenols and other products of putrefaction is increased by a diet favorable to the growth of putrefactive bacteria and by constipation. Since yeast is said to influence both the type of intestinal flora and constipation, there should be a decreased absorption of putrefaction products when yeast is ingested.

The methods for quantitative estimation of indican are very unsatisfactory so we limited ourselves to the determination of total urinary phenols. The method of Folin and Denis was used.

The daily averages of phenol excretion for the control, yeast and after-periods respectively were as follows: E. S., 0.60 mgm., 0.523 mgm., 0.516 mgm.; E. K. 0.353, 0.351, 0.446; Z. L. 0.292, 0.323, 0.253; H. W. 0.313, 0.245, 0.193; O. H. 0.451, 0.348, 0.252; G. H. 0.402, 0.293, 0.314.

It is obvious from these figures that the average excretion of phenols during the yeast period was noticeably less for four individuals, the same for one individual and increased for one. The two latter subjects were the only ones of the group who failed to have daily evacuations. In the after period the total daily excretion of phenols was still less than in the yeast period for three of the subjects who showed a decrease during the yeast period. For the fourth subject the excretion of phenols during the after period was 0.021 gram higher than during the yeast period, but still 0.088 gram less than for the first control period. The one individual who was very constipated and who showed an increase of 0.031 gram phenols for the yeast period fell 0.07 gram during the after period. This value was also 0.04 gram less than for the control period. Only one of the six subjects showed an increase in the after period. There was then, in five subjects, a distinct drop in phenol excretion during either the yeast period or the after period. In three of these there was a continuous drop in both the yeast and after periods. When we consider that the yeast introduced an additional 0.112 gram (5) of tyrosine daily as a possible source of phenols the influence of the yeast in decreasing phenol excretion is pronounced. Since this decrease in phenol excretion continued in the after period and occurred even in the very constipated individual, the probable interpretation of the results is that yeast changes the intestinal flora.

Murlin and Mattill (4) found the urinary output of phenols increased

during the yeast period, but not by an amount equal to the tyrosine derived from the yeast. They also found that the increase in phenols was less on six cakes of yeast daily than on one and one-half cakes. Their subjects were on a high protein diet which included meat, a diet very favorable to putrefactive bacteria. Also their yeast periods were in no cases longer than four days. The subjects of this experiment, on the other hand, were on a low protein, non-meat diet and the yeast period was extended over eight

TABLE 3

Phosphorus

First experiment

	PERIOD	URINE	FECES	TOTAL EXCRETION	TOTAL BLOOD
E. S.	Control	0.83	0.645	1.475	37.9
	Yeast	0.685	0.99	1.675	37.8
	After	0.835	0.511	1.346	39.1
E. K.	Control	0.639	0.372	1.011	40.0
	Yeast	0.610	0.527	1.137	34.9
	After	0.65	0.403	1.053	37.0
Z. L.	Control	0.59	0.622	1.212	33.8
	Yeast	0.69	0.899	1.589	34.5
	After	0.513	0.757	1.270	34.0
H. W.	Control	0.696	0.56	1.256	33.8
	Yeast	0.72	0.33	1.05	38.6
	After	0.711	0.619	1.33	34.0
O. H.	Control	0.820	0.583	1.403	37.0
	Yeast	0.872	1.23	2.102	40.57
	After	0.734	0.766	1.50	35.4
G. H.	Control	0.639	0.536	1.175	40.2
	Yeast	0.591	0.505	1.096	34.4
	After	0.574	0.497	1.071	35.6

days. Under these conditions the change in intestinal flora to a non-putrefactive type could be more readily effected.

Phosphorus. Since yeast has a high phosphorus content it was of interest to observe how this was eliminated. In the first experiment total blood, urinary and fecal phosphorus were determined. Of the six individuals on the experiment four showed appreciable increases in fecal phosphorus during the yeast period (table 3). The total weight of feces from O. H. for the first analysis of the yeast period was not recorded. However, the phosphorus had been run on duplicate samples and was 9.5 mgm.

per gram of moist feces as compared with 9.1 mgm. per gram moist feces for the second interval of the yeast period. Since his evacuations were regular and his average daily weight of moist feces was in every other case close to 100 grams, we are justified in assuming an average daily fecal phosphorus of 1 gram or more during the yeast period. Z. L. who showed no increased phosphorus elimination was exceedingly constipated during the experiment and had a marked diarrhea with considerable distress from gas the day following the close of the experiment. It is possible that with the marked constipation that existed in this individual a complete removal of residue from the yeast period was not possible until the diarrhea developed. In this case an analysis for that period might have accounted for the extra phosphorus.

G. H. suffered from indigestion at the beginning of the experiment. His urine was low in volume and extremely turbid, a heavy precipitate settling out even at room temperature. As the experiment progressed his daily urinary and fecal phosphorus decreased irrespective of yeast. This may have been the result of a radical change during this experiment from his usual dietary habits. In all individuals there was a distinct tendency for the urinary phosphorus to increase when total blood phosphorus was high. Although in two cases the total blood phosphorus was increased during the yeast period, there were two individuals in which it was distinctly lower and two in which it remained the same. For the most part the yeast phosphorus seemed to be eliminated promptly through the feces. In only one case, H. W., was there a noticeable rise in urinary phosphorus during the yeast period.

Glucose. Earlier workers have suggested repeatedly that yeast ingestion influences carbohydrate metabolism. For this reason and also to see whether the pentoses of the yeast itself are absorbed and excreted through the urine, we determined blood and urinary reducing sugars. The Folin-Wu, the Folin, and the Somogyi modification of the Shaffer-Hartman methods were all used. The two latter methods gave lower values but paralleled fairly well those obtained by the Folin-Wu method except in two or three individual determinations on urine.

There was distinctly an individual variation in the urinary sugar which corresponded roughly to the creatinine value.

After several weeks of yeast ingestion with a low protein diet, the blood sugar values were somewhat higher than for the non-yeast periods. This was also true in general for the urine. However, the change was not sufficiently marked nor consistent to warrant any conclusions. On the high protein diet the urinary sugar and blood sugar were higher for both individuals than was the case on the low protein diet. This may have followed the change to high protein or resulted in part from the long-continued (three months) yeast ingestion.

Moisture in feces. The moisture content of the feces was not materially affected nor consistently so by the addition or elimination of the yeast in the diet. In experiment I the feces from E. S., E. K., O. H. and G. H. contained 1.2 to 1.7 per cent less moisture during the yeast period than in the control period. In the after-period without yeast, in E. S. and E. K. the moisture again increased by 3.2 to 5.0 per cent whereas in O. H. and G. H. it continued to decrease by 0.9 to 1.45 per cent. The other two individuals reacted very differently. H. W. showed no change from the control to the yeast period and an increase of 3.8 per cent moisture in the after-period. Z. L. showed a marked lowering in moisture content (8.3 per cent) in changing from the control to the yeast period and again a rise of 4.5 per cent in the after-period.

In the second experiment the four subjects showed increases of moisture from 0.8 to 6.0 per cent when changed from the control to the yeast period. In the third experiment the two subjects reacted exactly the opposite. E. S. showed a lowering by 4.1 per cent and E. K. an increase of 11 per cent moisture when changed from the yeast to the after-period.

In general the indications are that yeast has no important rôle in influencing fecal moisture because there were variations in moisture content of different samples from the same individual and the same period as great as were found in analyses from different periods. Since the subjects noted easier evacuations following ingestion of yeast we believe this is due to a greater porosity of the feces as a result of fermentation. This was noted by one of us in preparing samples of feces for analysis—the yeast feces could be mixed much more easily than the control feces even though the moisture per cent was the same.

The reaction of the subjects to the yeast varied considerably. In some cases it was eaten raw like cheese either alone or on crackers or bread. Others took it suspended in cold water. Some complained of the after taste of the yeast and the discomfort from gas and belching. These annoyances became less noticeable after a week or ten days and especially so if the yeast was taken *before* the meal. It certainly appears that either the yeast is better digested or some other adjustment takes place after one becomes accustomed to taking it.

SUMMARY

In this study an attempt was made to place subjects on controlled diets, first a low purine, low protein and second a high protein, meat containing diet for a sufficiently long period to obtain several sets of analyses on each period. The effect of adding yeast to these diets was also determined.

Briefly the results are as follows:

1. The addition of yeast to a low protein, low purine diet after the subjects had arrived at a low uric acid elimination caused no increase in uric

acid excretion. If, on the other hand, the yeast was added while the uric acid elimination was still high or while subjects were on a high protein, meat diet an increased elimination of uric acid followed promptly. When yeast was discontinued the uric acid nitrogen fell off rapidly.

2. There was no increase in blood uric acid even after several weeks of yeast ingestion.

3. The total urinary phenols were less during and following yeast ingestion in five out of six individuals. This evidence of decreased intestinal putrefaction suggests that yeast may have a favorable action toward a change in the intestinal flora.

4. There was during yeast ingestion an increase in total (urinary and fecal) nitrogen and phosphorus. The larger share of this excess excretion was found in the feces.

5. While yeast generally produced greater regularity and ease of evacuations, the moisture content of feces did not seem to be noticeably or consistently increased. The yeast feces were more bulky and easier to mix and sample. This change in the character of the feces was probably the result of yeast fermentation and greater porosity.

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We wish also to express our appreciation of the many helpful suggestions of Dr. F. C. Koch, the cheerful coöperation of those who acted as subjects for the experiment and the technical assistance of Zonja W. Lawrence and G. F. Cartland.

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THE ENERGY FACTOR IN RELATION TO FOOD INTAKE: EXPERIMENTS ON THE DOG

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"Rubner says that many years of experience with dogs leads him to believe that appetite and capacity for digestion and absorption depend on the dog's requirement for energy in his given state of nutrition. A diet which a dog will greedily devour when in a room at a temperature of 0°, he will in part refuse when at a temperature of 33°."[1]

The literature concerning energy metabolism is quite sufficient to convince anyone that throughout the long gamut of bodily activities most of which occur in cycles—from day to day or week to week—there must be an adjustment of energy intake to energy needs. Numerous levels of such energy adjustments for men in different occupations have been determined and are recorded in textbooks of nutrition.

In an earlier paper (1923) the writer discussed a new method of feeding dogs artificial mixtures of isolated foodstuffs. The relation of vitamin B to appetite was emphasized. Attention was also called to the importance of controlling the influence of the energy factor on the desire to eat by feeding only enough calories for maintenance of the body weight. In this connection an important question arises. What can be taken as representing the ideal or optimum state of nutrition in the dog? It is quite possible that an investigator using the writer's method of feeding as described would be keeping his animal at a constant body weight far below the optimum and therefore really be working with a much undernourished dog.

Another question of interest presents itself. Consider a group of dogs living under reasonably constant laboratory conditions, ingesting measured amounts of a standard artificial diet of known caloric content. If a second food mixture of greater energy content be fed, will these animals eat less of it than the first? Will the food intakes of the two rations in grams be to each other inversely as their respective energy contents? Observations of food intake in rats by Osborne and Mendel 1919) and Smith and Carey (1923), and consideration of man's dietary habits certainly suggest a positive answer. The importance of further experimental tests

[1] Quoted by Lusk (1917, page 344) from Rubner (1902).

of the question will be realized more fully when one considers that some investigators in the field of nutrition have attempted to draw what has seemed to others to be unwarranted conclusions from experiments, in which the energy value of the ration was ignored and no records of food intake were kept.[2] The answers to these questions, furthermore, have a bearing on the principles and practise that should be followed in feeding individuals who do not have an opportunity for exercise of free choice of food—domesticated animals and babies, for example.

The writer has conducted feeding experiments with dogs over a period of several years with the view to obtaining data which should prove useful in answering some of the questions stated above. This paper aims to describe these experiments, the results obtained, and some of the applications of the findings that have been made in this laboratory.

METHODS. *Plan of experiments and calculations.* The scheme of experimentation was to offer the dog an amount of food greater than that estimated to be required or likely to be eaten, leaving the ration in the cage about three hours, then removing it, weighing and determining the amount actually consumed. Some difficulty was experienced at first in estimating the amount of food to be offered daily. In every case the initial response of the animal over a week or more was to eat all of the food offered. Whenever this happened, the amount of food offered was increased at intervals until it had become so large that the animal could become gorged, with subsequent vomiting. In such cases it was necessary to disregard all food intake data prior to this occurrence—see details of the method of calculation—unless, as sometimes happened, the dog reingested the vomitus.

The animal was always weighed just before being offered food. Data concerning body-weight and food intake were secured for *every day* of the experiment. Weighings were to the nearest ten grams. With dogs weighing ten kilos or more, this constitutes weighing to the fourth significant figure and represents an accuracy greater than is theoretically allowable for measurements of this kind. In using these weights for calculations the fourth figure was rounded out to give three significant figures. With dogs weighing less than ten kilos the three significant figures obtained directly from the daily weighings were used for calculation purposes.

Following this preliminary period characterized by steady increase in body weight, there occurred the period of equilibrium or adjustment characterized by fairly wide daily variations in food intake concomitant with only slight changes in body weight. During this period the dog was regarded as having come into a state of adjustment of its food intake to the bodily needs.

[2] Consider the controversy between McCollum and his associates and Osborne and Mendel (1918 a) with respect to the nutritive value of some of the cereal grains.

In order to calculate the energy level thus established, as many periods as possible ranging from eight to thirty or more days in length were taken,[3] the criterion of an adjustment period satisfactory for purposes of calculation being that the body weights on the first and last days should be approximately equal. If the weight had increased after the first day due to retention of food in one form or another, this retained material had been utilized by the end of the period. Conversely, if the animal had lost weight after the first day the loss had been made up by the last day. In the majority of periods calculated the differences between the first and last body weights were not greater than twenty grams. A few cases where the differences were of the order of thirty or forty grams were also calculated. The weight of the excreta and doubt as to whether they had been eliminated just before weighing make such a difference or an even larger one insignificant.

This method of calculation seemed to be as good as any other that could be devised. It does not obviate the error that changes in body weight may be due to water retention or loss. Such an error is probably not significant in the experiments where the same food was eaten daily; where the adjustments made to different diets were compared, it may account for some of the differences noticed.

In using this method one must be careful to avoid using periods that are too short. Just how few days this means proved difficult to decide. Examination of the voluminous data obtained and consideration of the behavior of the animals led to the decision to regard eight days as the shortest period deserving consideration. It may be contended by some that only the longest periods ought to be used for the calculations, particularly if one follows Sherman's (1924) discussion of the shortcomings of methods which endeavor to estimate metabolism by changes in body weight. To this, however, the writer does not agree.

Inasmuch as the arbitrary selection of any one period as defined above occasions some error, attempts were made to evaluate it. As many periods as possible were collected and their data treated statistically. Detailed data pertaining to dog 70 are presented in table 1 and serve to illustrate not only how periods were selected from the protocols but how they were treated statistically.

Foods. The first adjustment trials were made using diet III shown in table 2. Data were obtained from twelve dogs fed this diet. Later, four of these animals were fed other diets of greater calorific content and adjustment levels determined. The details of the other diets used are given in tables 3 and 4. Tables 2, 3, and 4 are given in the Appendix.

[3] Dog 69 maintained an almost constant body weight over an adjustment period of sixty-one days.

TABLE 1

*Dog 70**

LENGTH OF PERIOD		BODY WEIGHT		FOOD CONSUMPTION		CALCULATIONS	
From — to — days as given in protocol	Total length	From — to — kilos as given in protocol	Average	Food and vitavose	Food × 4.9 C. Vit. × 4.18 C.	Per day	Per kilo per day
	days		*kgm.*	*grams*	*Calories*	*Calories*	*Calories*
				1,604			
4–20	17	5.85–5.84	5.82	69.5	8,145	479	82.3
				911			
14–22	9	5.96–5.98	5.88	36	3,612	401	68.2
				968			
8–18	11	5.80–5.79	5.82	47	4,936	449	77.1
				1,242			
4–16	13	5.85–5.84	5.82	47.5	6,279	483	83.0
				1,319			
7–20	14	5.85–5.84	5.82	60	6,709	479	82.4
				1,332			
5–19	15	5.85–5.82	5.82	60	6,773	452	77.6
				1,160			
7–19	13	5.85–5.82	5.82	56	5,914	455	78.2
				956			
9–19	11	5.80–5.82	5.82	51	4,894	445	76.5
				1,153			
8–20	13	5.80–5.84	5.82	55	5,875	452	77.7
Total number of periods = 9.....		Average	5.82			455	78.1

Calories per kilo per day: average	78.1 Cal.
Standard deviation	4.3 Cal.
Standard deviation in per cent of average	5.5 per cent
Probable error of average	0.97 Cal.

* At new laboratory. See table 5.

Some experiments were made using diets containing fewer calories per gram than diet III but with only partial success. It proved difficult to make a low-calorie diet that dogs would eat with any relish, such mixtures being too powdery. The writer could not satisfy himself that those animals which did eat some of these powdery rations were really in a state of adjustment, therefore calculations covering such periods were not made. In planning these diets the amounts of protein, salt and

vitamins decided upon were such that wide variation in daily food intake could occur without the intake of these essentials falling below the physiological minima over any fairly extended period.

Food was weighed to three significant figures. When Yeast Vitamine Powder (Harris)[4] was used, the amount given was so small that its energy content could be ignored. When Vitavose[5] was fed as a source of the antineuritic factor, its calorific content was calculated on the assumption that the material is carbohydrate. A published analysis of this material (Hoffmann, 1925) shows that this assumption involves no serious error.

Additional data of interest. Feeding trials carried out on the first six dogs were made before satisfactory formulae for estimating the body surface area of dogs were available. Some attempts were made to estimate the area by a system of linear measurements similar to that employed by Rubner (1883) but the results obtained differed so much from those calculated by Rubner's formula ($S = 0.112\ W^{2/3}$) and the whole procedure seemed so inaccurate that the figures were rejected. Subsequently the problem of estimating the surface area of dogs was attacked with the results reported elsewhere (Cowgill and Drabkin, 1927), in the course of which work there came a realization of the importance of such additional data as the body length —tip of nose to anus—and the *Nutritive Index* based on von Pirquet's (1917) *pelidisi* principle, namely $\frac{W^{1/3}}{L}$. This index was also determined for many dogs in obviously quite different states of nutrition but not being used for these caloric adjustment studies. The highest value ever obtained for this index was 0.34 yielded by an extremely obese animal. A greatly emaciated dog gave a value of 0.26. For further discussion of this index the reader is referred to the surface area paper of Cowgill and Drabkin (1927).

In table 7 it will be noticed that the nutritive index value for dogs that have voluntarily adjusted and arrived at a good state of nutrition is 0.30. This figure acquires added interest, when it is realized that the group of animals represented in table 7 included dogs of the long-lean, short-stout, long-haired and short-haired types. The agreement of the individual values is striking and contrasts considerably with the values yielded by the seven fat, average and lean dogs, whose surface areas were measured by Drabkin and the author.

A change of laboratory environment as a test of the accuracy of the method. If our method of studying the problem previously outlined is sound, a change of laboratory surroundings without any changes in the diet should not produce any marked difference in the adjustment of the dog to the single diet offered. An opportunity for testing this point presented itself when the Department of Physiological Chemistry moved from the old laboratory to quarters in the new Sterling Hall of Medicine. Adjustment data pertaining to the old laboratory had just been obtained with three dogs (69), (70), (71). Before beginning experiments with diets of higher caloric content it was considered worth while to repeat the work in the new laboratory. Table 5 contains a resumé of the two sets of experiments,

[4] From the Harris Laboratories, Tuckahoe, New York.

[5] From the Ward Baking Company, New York. Now obtainable from E. R. Squibb & Sons, New York.

and shows quite clearly that the adjustments made by the animals in different quarters were the same. Dog 69 showed the greatest apparent lack of agreement, but the difference is not significant, its magnitude being of the order of the standard deviation. The agreement in the cases of the other two animals is quite remarkable.

RESULTS. *Data: Tables and chart.* The data are summarized in table 6. As has already been stated, some observations had been made on six dogs before the new surface area formula became available. The body lengths of these animals were not measured. Inasmuch as the nutritive conditions of all of the animals under adjustment conditions may without appreciable error be assumed to be the same—see column 5 in table 7—the body surface areas of all the dogs experimented with and listed in table

TABLE 5

A comparison of the caloric adjustments to the same diet but in different laboratories

DOG	NUMBER OF PERIODS USED IN CALCULATIONS	BODY WEIGHT MEAN	CALORIES PER KILO PER DAY			PLACE
			Mean	Standard deviation in per cent of mean	Probable error of mean	
		kgm.				
69	15	3.41	99.3	7.2	1.25	Old laboratory
	16	3.56	91.9	7.5	1.16	New laboratory
70	61	6.38	77.4	4.0	0.27	Old laboratory
	9	5.82	78.1	5.5	0.97	New laboratory
71	5	14.9	65.4	0.76	0.15	Old laboratory
	5	15.0	65.6	4.3	0.85	New laboratory

6 were estimated by Rubner's formula. The values for the calories per square meter per hour thus obtained were used for purposes of comparison.

It should be borne in mind that the Rubner formula gives surface area values slightly greater than those yielded by the new formula (Cowgill and Drabkin, 1927). The values for the calories per square meter per hour will therefore be somewhat smaller when Rubner's formula is employed. Inasmuch as absolute values are not of as great importance here as the agreement or lack of agreement of the data for different animals, inability to use the new formula with all the dogs listed in table 6 is of little consequence.

Length measurements as well as body weights were obtained with the last six dogs experimented upon, and the body surface areas estimated by the new formula. The nutritive index was also evaluated. Table 7 has been prepared with the view to presenting these as well as other data pertaining to these six animals. Standard deviations and probable error

values are shown for the calories per kilo per day for each dog (columns 11 and 12) as well as for the calories per square meter per hour for the group. It is thus possible to obtain some idea as to the variation shown by each dog as well as the variation exhibited by the animals considered as a

TABLE 6

DOG	BODY WEIGHT	BODY SURFACE AREA (RUBNER)	CALORIES				DIET, ETC.
			Per hour	Per day	Per kilo per day	Per hour per sq. m.	
	kgm.	*sq. m.*					
69	3.41	0.254	14.1	338	99.2	55.6	III. Old lab. Long-haired
69	3.56	0.261	13.6	326	91.6	52.1	III. New lab.
58	4.30	0.296	18.9	454	105.5	63.8	III. Old lab. Short-haired
61	4.40	0.301	18.2	437	99.3	60.5	III. Old lab. Short-haired
76	5.04	0.329	18.3	439	87.1	55.6	III. New lab. Short-haired
76	5.57	0.352	24.0	575	103.3	68.2	XVIIb. New lab.
66	5.40	0.345	24.2	581	107.5	70.2	III. Old. lab. Short-haired, quite active
70	5.82	0.362	19.0	455	78.1	52.5	III. New lab. Long-haired
70	6.38	0.385	21.2	509	79.7	55.0	III. Old lab.
77	7.94	0.446	25.8	620	78.1	57.9	III. New lab. Short-haired
77	8.40	0.463	27.3	654	77.9	57.7	XVb. New lab.
62	8.57	0.469	36.8	883	103.0	78.5	III. Old lab. Short-haired very active
75	12.0	0.587	36.0	851	71.9	61.3	III. New lab. Short-haired
75	12.5	0.603	35.5	863	68.1	58.9	XVIIb. New lab.
64	13.5	0.635	44.1	1058	78.4	69.5	III. Old. lab. Short-haired, very active
67	14.9	0.678	40.6	974	65.4	59.9	III. Old lab. Short-haired
71	15.0	0.681	40.9	980	65.3	60.0	III. New lab. Short-haired
71	15.4	0.693	39.1	948	61.6	56.4	XVIIa. New lab.

Calories per hr. per sq. m. mean	60.8	
Average deviation	5.2	
Average deviation in per cent of mean	8.6	per cent
Standard deviation	6.7	
Standard deviation in per cent of mean	11.1	per cent
Probable error of mean	1.6	
$\frac{\text{Standard deviation}}{\text{Average deviation}}$	1.29	

group. The experiments with diets having different calorific contents were performed on four of these animals.

Figure 1 was prepared from the data contained in table 6 and those yielded by twenty-eight cases where dogs were fed a maintenance ration designed to be complete and to be entirely consumed voluntarily every

TABLE 7

DOG	BODY WEIGHT AVERAGE	BODY LENGTH	BODY SURFACE AREA	NUTRITIVE INDEX*	NUMBER OF PERIODS USED IN CALCULATIONS	CALORIES						DIET†
						Per day	Per hour	Per square meter per hour	Per kilo per day			
									Average	Standard deviation in per cent of average	Probable error	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	kgm.	*cm.*	*sq.m.*									
69	3.56	50	0.227	0.305	16	326	13.6	59.9	91.6	7.51	1.16	III 1 gm. = 4.88 Calories
70	5.82	61	0.332	0.295	9	455	19.0	57.1	78.1	5.50	0.97	III
76	5.04	57	0.295	0.301	10	439	18.3	62.0	87.1	3.24	0.76	III
	5.57	57	0.306	0.311	8	575	24.0	78.3	103.3	13.30	3.12	XVIIb 1 gm. = 6.10 Calories
77	7.94	67	0.409	0.297	13	620	25.8	62.9	78.1	12.10	1.27	III
	8.40	67	0.418	0.303	8	654	27.3	65.2	77.9	6.00	1.12	XVIIb
75	12.00	78	0.553	0.294	8	863	36.0	65.0	71.9	9.10	1.57	III
	12.50	78	0.564	0.298	15	851	35.5	62.9	68.1	3.66	0.42	XVIIb
71	15.00	78	0.602	0.316	5	980	40.9	67.9	65.3	4.32	0.85	III
	15.40	78	0.610	0.319	7	948	39.1	64.0	61.6	8.32	1.27	XVIIa 1 gm. = 6.06 Calories
Average				0.304				64.5		7.31		

Calories per hour per square meter: Average 64.5
Average deviation 3.66
Average deviation in per cent of mean ..... 5.67 per cent
Standard deviation 5.39
Standard deviation in per cent of mean ..... 8.36 per cent

* $\frac{W^{1/3}}{L}$ (Cowgill and Drabkin, 1927).

† The caloric value for diet III given in this column is based on actual utilization studies. The values for the other diets listed assume a utilization of 95 per cent of the energy.

day. The last named group of animals included those used by students and research workers in this laboratory.[6]

Energy factor regulates intake of "complete" food: caloric adjustment. The data summarized in these tables and charts indicate clearly that a healthy adult dog tends to regulate the amount of adequate food eaten so as to maintain constant body weight, constant state of nutrition as measured by the nutritive index adopted, and a constant intake of energy. This condition of equilibrium the writer has chosen to call one of *caloric adjustment.* Before proceeding to a more detailed consideration of the caloric adjustment data it is pertinent to inquire whether the adjustments exhibited by the animals may not have been made to some other factor in nutrition, protein for example, instead of energy. Extensive calculations were made of the amounts of various nutrients consumed during the periods of adjustment and referred both to body surface area and body weight. Mere inspection of most of the figures reveals serious lack of agreement among the animals as to their consumption of the foodstuffs in question. Lack of space forbids giving the results of these numerous calculations in detail. However, a summary in the form of a comparison of the average deviations exhibited with respect to the various nutritive factors ingested is presented in table 8. This shows quite clearly that *calories per unit of body surface area constitute the common denominator of adjustments: 1, of the same dog to different complete diets as well as 2, of those of different size dogs to the same and to different complete rations.*

On the basis of the figures in table 8 it might be argued, perhaps, that the animals adjusted to roughage and salt mixture almost as well as to calories. The writer does not believe that students of nutrition will contend seriously for this view. In planning the different diets the amounts of salt mixture and roughage selected were such as to insure over any appreciable period an intake well above the minima required for satisfactory nutrition. In constructing diets richer in calories than the standard ration (III) the amounts of salt mixture and roughage to be included were increased. Therefore an adjustment of the animal to calories would have resulted in a simultaneous and similar adjustment to salts and roughage. Wide variation in intake of protein, carbohydrate and fat referred to body surface area and body weight occurred. The adjustments made therefore were *not* governed by these important foodstuffs. Vitamin B is not listed in the table because the amounts of yeast vitamin (Harris) or wheat germ preparation (Vitavose) fed daily were always at the rate of 40 and 600 milligrams per kilogram body weight respectively,

[6] The author desires to thank the following persons for their kindness in submitting data: Doctors H. J. Deuel, Jr., Marion Fay, G. A. Williams, D. L. Drabkin, L. R. Cerecedo and C. M. McCay.

doses that had been determined by previous research to be minimal (Cowgill, Deuel and Smith, 1925).

Therefore the use of the term *caloric adjustment* to describe the condition that prevails in animals allowed to choose for themselves the amounts of a "complete" food to be eaten is justified.

The significance of this phenomenon called *caloric adjustment* is more readily appreciated when considered in relation to the feeding of diets having different energy contents. The levels at which dogs of different sizes establish a caloric adjustment possess considerable interest, whether the adjustment be referred to body surface area, body weight or to body length.

TABLE 8

The extent of agreement with respect to various nutritive factors of dogs listed in table 7, when adjusted to different diets, may be shown by the figures for the average deviations in per cent of the respective means. A value of approximately 10 or over indicates decided lack of agreement.

NUTRITIVE FACTOR CONSUMED DURING PERIOD OF ADJUSTMENT	AVERAGE DEVIATION IN PER CENT OF MEAN	
	Per square meter per day	Per kilo per day
	per cent	*per cent*
Calories	5.7	12.0
"Roughage"	7.0	13.9
Salt mixture	7.0	10.1
Vitamin A (butter)	11.4	14.8
Carbohydrate as such in food	14.2	19.0
Available metabolizable carbohydrate (food carbohydrate + 0.58 protein)	19.2	23.1
Fat	19.4	16.2
Protein	27.2	33.0

Caloric adjustment to diets having different energy contents. In table 7 are presented the data obtained from four dogs (76, 77, 75 and 71), each of which adjusted first to diet III having a net utilized energy content of 4.88 calories per gram, and then to some other ration having a greater energy content. The writer is indebted to Dr. G. A. Williams for data taken from his utilization studies by which the net utilized energy value for diet III was calculated.

It will be noticed that each animal voluntarily adjusted to the high-calorie ration at a slightly higher body weight level. This may be the result of either a greater water retention associated with the high-calorie diet, or greater palatability of such a ration. Perhaps both factors contributed to the result. Concomitant with this increase in body weight all of the four animals under consideration, except dog 76, adjusted at a lower

level of calories per kilo per day. The differences are not great however and probably are not significant in view of the values of the corresponding standard deviations. In considering the large difference in the case of dog 76 in this respect, the high value of the standard deviation should be noted.

The adjustment values decrease with increase in body weight of the different animals, as would be expected in the case of basal metabolism

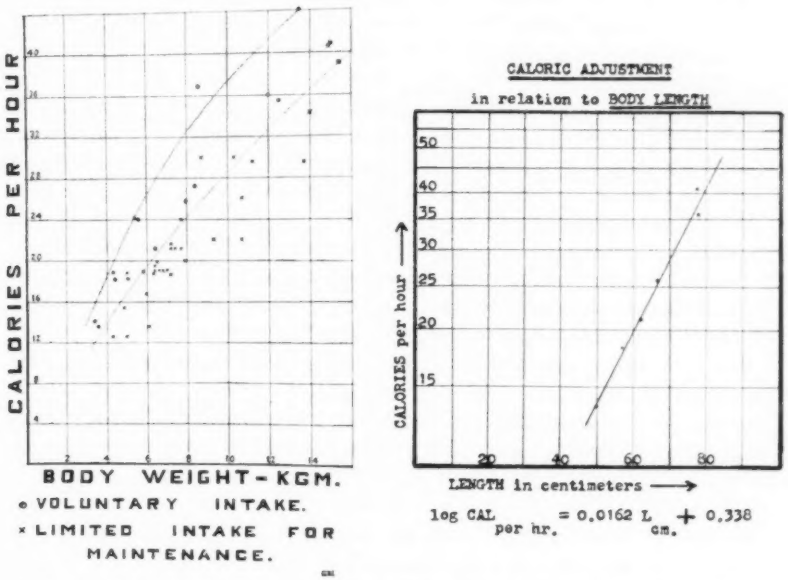
CALORIES PER HOUR
BODY WEIGHT - KGM.
• VOLUNTARY INTAKE.
× LIMITED INTAKE FOR MAINTENANCE.
CALORIC ADJUSTMENT
in relation to BODY LENGTH
CALORIES per hour
LENGTH in centimeters
log CAL per hr. = 0.0162 L cm. + 0.338

Fig. 1. Caloric adjustments of dogs are indicated by circles, whereas cases of limited intake designed to secure maintenance are represented by crosses. The area between the dotted lines may be regarded as representing the *zone of caloric adjustment*. In using this graph as an aid in determining at what level of caloric intake to feed a dog in order to secure voluntary daily consumption of all food offered and a good state of nutrition, it is important to avoid offering a number of calories for the body weight in question that would be represented by a plotted point lying within the zone of caloric adjustment. The aim should be to maintain conditions indicated by a point fairly close to but definitely below the lower curve.

from the work of Rubner (1883) and other investigators too numerous to mention individually.[7] This is true whether the comparison be of those figures obtained with diet III or of those yielded when the high-caloric rations were used.

Consider now the figures for calories per square meter per hour for dogs 71, 75, 76 and 77 in table 7, column 9. The standard deviation and prob-

[7] Krogh (1916) gives a good review of the work bearing on this point in chapter IX of his monograph.

able error values in columns 11 and 12 give some idea of the extent to which the values in column 9 are subject to variation. The agreement of these four animals with each other is very good with but one aberrant value, namely, that for dog 76, when fed the high calorie diet. The average of the standard deviations for these four dogs is 7.5 per cent. When it is borne in mind that the basal metabolism of a given individual measured under the most exact of conditions may vary as much as ten per cent (Benedict, 1915: Dubois, 1924), *the agreement of the total metabolism under "caloric adjustment" conditions of these dogs differing considerably in size and subsisting on diets differing markedly in energy content may be regarded as truly significant.*

It may be concluded, therefore, that dogs, when offered an abundance of an adequate ration, voluntarily adjust their food intake to their energy needs, as Rubner has suggested. This adjustment is such as to maintain a state of nutrition indicated by a nutritive index value of approximately 0.30. This adjustment, when obtained in cages under the conditions prevailing in this laboratory, is such that the animals maintain an intake of 64.5 calories per square meter per hour with an average deviation of 3.7 calories. It is very probable that caloric adjustment values for dogs confined to *metabolism* cages would be lower than this.

If it be assumed that the basal metabolism of the dog is approximately the same as man—an assumption that does not appear to be unwarranted (Cowgill and Drabkin, 1927)—then the total metabolism of the dogs listed in table 7 is comparable to a daily energy output of about 2800 calories for a man having a body surface area of 1.8 square meters.

Caloric adjustments in relation to body weight. Caloric adjustment observations had been made on six dogs before the measurements of body surface area and the development of accurate formulae therefrom were begun in this laboratory. The data from these animals together with those of the six dogs included in table 7 have been used to construct the chart in figure 1. Calories adjusted to per hour are plotted as ordinates and the body weights in kilograms as abscissae. Points representative of caloric adjustments are indicated by dots within circles. The two curves are drawn, the upper passing through points representing, with one exception, maxima of adjustment levels, whereas the lower curve passes through points representing the lowest levels of adjustment obtained. It is a curious and interesting fact that the points determining the upper curve correspond in every case to short-haired dogs, whereas the points fixing the lower curve with one exception refer to long-haired animals.

Each cross corresponds to a dog that was fed in accordance with the plan developed by the writer, in which the aim is to maintain a voluntary constant daily food intake. Some of the animals thus designated were fed by the author, but most of them were used by students and research

workers in this laboratory engaged on various problems in which, among other things, it was important to insure an unvarying daily voluntary intake of a purely artificial food mixture.

The plot of these data along with those of the caloric adjustment animals illustrates a practical point to be made concerning the control of the energy factor in relation to appetite and constant daily food intake. If, on an adequate diet, the energy need governs the animal's desire to partake of food, the obvious procedure to insure perfect food intake is to diminish the number of calories offered daily. To do this properly and at the same time keep the dog in a good state of nutrition, one must have some idea as to what level of intake the animal itself will select if given an opportunity. The area between the two curves may be regarded as representing the *zone of caloric adjustment.* It may serve as a rough guide for determining the level at which any particular dog would probably adjust voluntarily under the conditions prevailing at this laboratory. It will be noticed that a few of the crosses fall in this zone. The dogs here represented were, in the opinion of the writer, more likely to show an *apparent* loss of appetite due to adjustment to caloric needs, particularly during hot weather, than the other animals represented by the remaining crosses, because they were being maintained at a level very close to the one they themselves would have selected if given an opportunity.

Inasmuch as improvement in nutritive condition for an adult dog nearly always means increase in body weight, it is not easy to use the chart in figure 1 for prediction purposes. The data in table 7, however, enable one to construct tables of maintenance levels based upon considerations of caloric requirement 1, per unit of body surface area, and 2, per unit of body length.

Caloric adjustment in relation to body surface area. Reference to table 7 shows that under the conditions prevailing in this laboratory, dogs voluntarily adjust their food intake so as to insure a supply of approximately 64 calories per hour per square meter of body surface. The agreement in this respect of the animals quite different in size stands in considerable contrast to the caloric adjustment as related to body weight.

Use can be made of this fact to properly control the energy factor in relation to appetite, or the urge to eat. By measuring the weight and length of a given dog, the body surface area may be estimated from the nomogram published elsewhere (Cowgill and Drabkin, 1927). At the same time the nutritive condition of the animal will be determined. If the dog is quite thin, it will be advisable to improve its condition by a preliminary feeding period using a good diet. Conversely, if the animal is fat, as indicated by the value of the nutritive index on the nomogram, it should be fasted long enough to make the index value lie between the 1.1 and 1.2 diagonals on the chart.

Knowing the surface area and allowing 64 calories per square meter per hour, one can calculate the daily requirement of energy. The 64-calories value is subject to a standard deviation of 8.4 per cent. If, then, the calculated daily allotment of energy be reduced from 10 to 15 per cent, the resultant value should be outside—but not too much so—the zone of caloric adjustment to insure a good nutritive state as well as a *voluntary* daily ingestion of all the food offered even during periods of warm weather that are not too prolonged.

Another and perhaps easier way to attain the same end is to utilize the relationship demonstrated to exist between the caloric adjustment level and body length, about to be described.

Caloric adjustment in relation to body length. Benedict (1924) has emphasized the importance of considering the state of nutrition when studying metabolism, and has observed in this connection a very interesting relationship between body length and basal metabolism. The data in table 7 of this paper likewise show a relationship between the total metabolism at the caloric adjustment level and the body length. In figure 2 is shown the result of plotting on a logarithmic scale for ordinates the calories per hour against the body lengths as abscissae. The equation for the line drawn is also shown.

The writer hesitates to attach any peculiar significance to length in this relation, but chooses rather to regard the fundamental relationship as existing between calories and body surface area. The formula by which surface area is estimated includes length as one datum.

The equation of the line plotted in figure 2 can be used to construct a table useful in quickly estimating the caloric requirement at the adjustment as well as the maintenance level. The probable body weight of the dog, when in caloric adjustment, may also be determined. Such values have been calculated on the assumption that the nutritive index value will be 0.30. Table 9 was prepared by such calculations.

In constructing table 9 the values calculated from lengths greater than 78 and shorter than 50 really involve extrapolation. That the validity of such values may be questioned for this reason is freely admitted.

Discussion. It is certain that, when all of the dietary essentials such as protein, vitamins and salts are being provided in amounts sufficient to meet the bodily requirements, and there are no pronounced psychic influences operating, the energy need becomes the regulator of food intake. The question arises: does the energy need *always* govern the urge to eat? Are there any special conditions where other factors control the food intake?

In discussing these questions two important considerations should be borne in mind: 1, maintenance of the adult organism and growth of the young present somewhat different nutritive problems; and 2, freedom of choice in the selection of food is a factor.

TABLE 9

This table was prepared for use in quickly estimating from a given value of a dog's body length: (1) the daily energy intake under (a) voluntary caloric adjustment conditions, and (b) maintenance, where it is desired that the animal voluntarily ingest all of the experimental ration offered; and (2) the body weight which the animalwill probably have under caloric adjustment conditions.

LENGTH*	CALORIES			PROBABLE BODY WEIGHT AT ADJUSTMENT LEVEL WITH NUTRITIVE INDEX VALUE = 0.3
	At adjustment level		At maintenance level—per day‡	
	Per hour†	Per day		
cm.				
42	10.4	250	213	2.00
44	11.2	269	228	2.30
46	12.1	290	246	2.63
48	13.5	324	275	3.00
50	14.0	336	286	3.38
52	15.2	365	310	3.80
54	16.3	391	332	4.25
56	17.6	422	358	4.74
58	18.9	454	386	5.27
60	20.4	490	416	5.83
62	22.0	528	449	6.43
64	23.7	569	484	7.08
66	25.5	612	520	7.75
68	27.5	660	561	8.49
70	29.6	710	604	9.26
72	31.9	766	651	10.1
74	34.4	826	702	10.9
76	37.1	890	757	11.9
78	40.0	960	816	12.8
80	43.1	1034	879	13.8
82	46.4	1114	947	14.9
84	50.0	1200	1020	16.0
86	53.9	1294	1100	17.2
88	58.0	1392	1183	18.4
90	62.5	1500	1275	19.7
92	67.4	1608	1367	21.0
94	72.6	1742	1481	22.4
96	78.2	1877	1595	23.9
98	84.3	2023	1720	25.4
100	90.8	2179	1852	27.0

* From tip of nose to anus.

† From log Cal. per hour = 0.0162 L cm. + 0.338.

‡ 15 per cent below the adjustment level.

Vitamin B has been shown to be closely related to maintenance of the urge to eat in the dog (Karr, 1920; Cowgill, 1921). This phenomenon is of widespread significance as shown by the observations of numerous investigators on other species, such as the *rat* (Osborne and Mendel, 1917), *pigeon* (Klotz, 1926; Hoet, 1923; Kon 1927; Plimmer, Rosedale and Raymond, 1927), *cat* (unpublished observations of the writer), and *mouse* (Beard, 1926). The facts 1, that this relationship holds in the adult as well as in the growing animal, and 2, that it is exhibited when there are no other manifestations of vitamin deficiency, give to this dietary essential a peculiar significance, and elevate it to rank over the energy need as a regulator of appetite.

With respect to the other specific deficiencies the answer is not so clear. Anderson and Smith (1924) observed that the food intake of guinea pigs decreased with the onset of scurvy. Administration of vitamin C resulted in cure of the scurvy, increased food intake and resumption of growth. Examination of the food intake charts submitted and consideration of the behavior of the animals suggest that the decrease in food intake was rather definitely associated with the appearance of the scorbutic symptoms. In other words, the failure to eat became pronounced when the animal was seriously ill. One can hardly say, therefore, that the antiscorbutic vitamin may be a regulator of the urge to eat, if this regulative property becomes evident only when the vitamin-deficient condition is serious enough to result in clinical signs of scurvy.

Similarly the loss of appetite associated with vitamin A deficiency in the adult appears to be closely associated with the appearance of ophthalmological, cutaneous and other manifestations of disease. The vitamin is undoubtedly essential in such cases, and to that extent might be regarded as a regulator of the urge to eat, in that the organism's need for it initiates a hunt of the random sampling sort in an endeavor to remedy the deficiency.

It is the writer's present belief that under conditions of free choice and in the absence of pronounced psychic influences, deficiencies of any of the various dietary essentials usually operate sooner or later to initiate a search for the missing essential. A good example of this in adult organisms is seen in the *osteophagia* exhibited by cattle (Green, 1925). The organism may not know what is being searched for, but ingestion of various trial substances with physiological well-being following the use of the required food factor, soon results in a helpful food habit and a good appetite. In all probability man's development of the habit of consuming cod liver oil, lime juice, etc., long before vitamins were known rests on such a basis. Under conditions of sufficiently limited choice, lack of certain essentials results in loss of the urge to eat, either quite early before any other symptoms are evident—vitamin B, for example—or later, when the deficiency has resulted in making the animal definitely ill as shown

by eye diseases, scurvy, or some other manifestation. All such cases might be designated as *true* losses of appetite, in contrast to the *apparent* loss due simply to adjustment of the organism's energy intake to its energy needs.

There are several important applications of the principle of caloric adjustment that should be emphasized. In the field of experimentation in nutrition and the planning of experimental diets, care should be taken to see that the ratios of salt content and other essentials to the total calories are such that the adjustment of intake to calorific value of the total food mixture can be made without rendering the consumption of the essentials below the minimum necessary amounts. Powers (1925) has shown what happens when this principle is applied to various milk mixtures used in feeding infants. To some it may appear unnecessary thus to reiterate what has already been emphasized by Smith and Carey (1923) and Osborne and Mendel (1918b). However, the continued appearance of papers reporting feeding experiments with deductions therefrom but neither giving evidence that records of food intake were kept nor showing that the energy value of the ration was considered, together with a widespread lack of appreciation of this important principle constitute sufficient justification.

It is the practise of some pediatricians to regulate arbitrarily according to some so-called "standard" the amount of milk or milk-mixture that infants shall receive. One widely recommended manual of advice to mothers (Holt, 1918) cautions against overfeeding the infant. Undoubtedly the amount of food to be given a baby must be considered in relation to the well-being of the alimentary tract and other conditions, but it is a bit difficult to understand why, in the absence of gastro-intestinal trouble or other imperative reasons, there should be any conscious restriction of food, and that the infant should not have all the nourishment it desires. The better part of wisdom for such pediatricians would seem to be to place emphasis on giving all that the baby wishes and restricting the food only when definite indications for it appear. Furthermore, a distinction should be made between overfeeding with respect to volume of fluid that the stomach can hold without regurgitation, and overfeeding with respect to the fundamental nutrients such as protein, calories, etc. If caution is taken to insure that the food is "complete" and fed in appropriate concentrations, it is the writer's belief that the healthy infant may be relied upon to govern the amount of his food intake wisely according to the principle of caloric adjustment.

SUMMARY—CONCLUSION

Twelve adult dogs ranging from 3.41 to 15 kilograms body weight were fed all that they wished of a "perfect" artificial diet and the amounts of

food eaten were carefully measured. The first reaction to these experimental conditions in every case was complete ingestion of all food offered concomitant with an increase of body weight until a certain weight different for each animal was reached. Thereafter the food intake varied widely from day to day, but the body weight remained remarkably constant.

Data for estimating body surface area as well as the value of the nutritive index were obtained from six dogs of the series.

Four dogs were then offered daily measured amounts of another artificial diet having a much greater energy content. Under these conditions they ate fewer grams of the ration but *approximately the same number of calories per day* as formerly. This condition of the animal characterized by voluntary maintenance of constant body weight and nutritive state has been designated that of *caloric adjustment*.

When the energy intake of all of the dogs under caloric adjustment conditions is considered in relation to body weight, it is found that, just as is the case with basal metabolism, the smaller the animal, the greater is the total metabolism per unit of weight.

Considered in relation to body surface area, the caloric adjustments made by six dogs ranging from 3.41 to 15 kilograms body weight were approximately the same, namely, 64 calories per hour per square meter, with a standard deviation value equal to 8.4 per cent. In other words, the total metabolism values for these dogs of different sizes were in quite constant relation to their body surface areas. The variation was approximately the same as that shown by the basal metabolism of a normal individual.

A linear relationship was found to exist between the body length and the logarithm of the calories per hour adjusted to by the animals. On the basis of this relationship a table was constructed by which one may quickly estimate from a given length the caloric intake at the adjustment level, as well as at a level suitable for maintenance of a voluntary daily ingestion of all the food offered, and a good nutritive state under the conditions prevailing in this laboratory.

The bearing of these findings on the technic of experimentation in the field of nutrition and in relation to the dietary habits of man and animals was discussed.

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APPENDIX

The details of the experimental diets used are presented in tables 2, 3 and 4.

TABLE 2

Diet III

	GRAMS	CALORIES	PERCENT	
Casein commercial (12.7 per cent N)	6.3	20.6*	41.2	
Sucrose	4.5	18.0	29.4	
Lard	2.8	25.2	18.3	25.5
Butter	1.1	9.0†	7.2	
Bone ash	0.4		2.6	
Salt mixture‡	0.2		1.3	
Total	15.3	72.8	100.0	

This kilo-unit contains 0.8 gram of N and 73 calories, 46.5 per cent of which are furnished by fat.

* Calculated on a basis of 81.9 per cent protein.

† Figured as containing 90 per cent fat.

‡ Used by Karr (1920).

TABLE 3

Diet 17a

	GRAMS	CALORIES*	PER CENT
Meat residue† (13 per cent N. 10 per cent fat, 82 per cent protein)	5.38	19.1 5.1	27.2
Sucrose	4.50	18.8	22.7
Beef tallow	7.00	66.2	35.5
Butter (10 per cent salt and water impurity)	1.99	17.0	10.0
Cellu flour‡	0.55		2.8
Salt mixture no. 2§	0.35		1.8
Total kilo-unit	19.77	126.2	100.0

This kilo-unit contains 0.7 gram N in protein of good quality and yields 6.38 Calories per gram.

* Loewy's factors are used, namely, 4.32 for protein, 4.18 for carbohydrate, and 9.46 for fat.

† From the Valentine Meat Juice Company, Richmond, Virginia.

‡ From the Chicago Dietetic Supply House, Chicago, Illinois.

§ Journ. Biol. Chem., 1923, lvi, 725 (see table III, p. 734).

TABLE 4

Diet 17b

	GRAMS	CALORIES*	PER CENT
Meat residue†(13 per cent N. 10 per cent fat, 82 per cent protein)	5.38	19.1 5.1	22.9
Sucrose	5.50	23.0	23.4
Beef tallow	7.00	66.2	29.7
Butter (10 per cent salt and water impurity)	2.25	18.9	9.6
Lard	2.00	18.9	8.5
Bone ash	0.80		3.4
Salt mixture no. 2‡	0.60		2.5
Total kilo-unit	23.53	151.2	100.0

This kilo-unit contains 0.7 gram N in protein of good quality and yields 6.43 Calories per gram.

* Loewy's factors are used, namely, 4.32 for protein, 4.18 for carbohydrate, and 9.46 for fat.

† From the Valentine Meat Juice Company, Richmond, Virginia.

‡ Journ. Biol. Chem., 1923, lvi, 725 (see table III, p. 734).

STUDIES OF MUSCULAR EXERCISE UNDER LOW BAROMETRIC PRESSURE

III. The Output of Carbon Dioxide

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In earlier papers (1925 and 1926) we have studied the changes of oxygen consumption, the oxygen debt, the frequency of breathing and the volume of respired air when work is done at various atmospheric pressures. In this paper we shall discuss the percentage and volume output of carbon dioxide and the changes in the respiratory quotient when work is done at barometric pressures of from 760 to 290 mm.

All experiments were conducted in a low pressure chamber from which the air was pumped in sufficient amount to reduce, and then to maintain, the barometric pressure within the chamber at any desired level. The arrangement of valves and exhaustion pump permitted a continuous flow of fresh air through the chamber throughout the entire period of experimentation. All work was done by young men on a Benedict-Cady bicycle ergometer, which is provided with four electromagnetic coils by means of which various degrees of resistance were obtained to provide for the needs of graded exertion.

The per cent of carbon dioxide in the expired air. The percentage of carbon dioxide in the expired air has been employed by Briggs (1920) to determine just which amount of work is an over-load. When exertion of increasing magnitudes is studied, it is found that the percentage of expired carbon dioxide at first rises and then falls. So long as the carbon dioxide percentage rises with added increments of work, the load is considered normal; when the percentage falls, it is regarded as an over-load; and when the percentage is at its maximum, it is a crest-load. It is believed that the fall in the percentage of exhaled carbon dioxide is associated with the escape of lactic acid from the muscles into the blood. Furthermore it is maintained that, up to the crest-load, the supply of oxygen is adequate for the oxidation and reconversion of lactic acid into its precursor and so only carbon dioxide escapes from the muscles into the blood, which it does in ever increasing amounts with added increments of work. However, beyond the crest-load the oxygen supply becomes inadequate, hence lactic

acid accumulates in the muscle and soon begins to escape into the blood. With the appearance of a deficiency in oxygen the process of reconversion is hampered and less carbon dioxide is formed. Hence the proportion of carbon dioxide exhaled will be diminished and this decrease, according to Briggs, gives a means of determining the size of the minimum over-load.

When man is placed under a reduced barometric pressure his available oxygen supply is decreased. We (1925) have shown that the oxidative processes of the body during work are retarded by a lowered barometric pressure. It would seem then, according to the above theory regarding load and over-load, that when one is called upon to do muscular work under a low barometric pressure, the size of the crest-load should be reduced.

With the expectation of determining a curve for the anoxemic changes in the size of crest-load we, early in our studies, determined the crest-load at various barometric pressures by a study of the carbon dioxide output. For this purpose we used five subjects at five barometric pressures that corresponded to those of altitudes of sea level, 10,000, 15,000, 20,000 and 25,000 feet. The size of the crest-load was found by increasing the load of work by increments of 2,000 foot pounds until the load became an over-load. The plan used was as follows: The subject was allowed to sit in the low pressure chamber for about half an hour, and if the experiment was to be made at a low pressure he remained seated until the desired pressure was reached. After five minutes at that pressure he moved onto the bicycle ergometer. After a lapse of two or three minutes the mouth-piece and nose clip were adjusted. Then three minutes later the collection of expired air in a Douglas bag was begun and continued for between five and ten minutes. Immediately after this the subject began to work on the bicycle, carrying a load of 2,000 foot pounds. After three minutes of work a second bag was connected with the expiratory valve and the expired air was collected for the next two minutes of work. A period of rest of fifteen or more minutes followed, the length of this period being determined by the time required for the return of the pulse rate and respiration to normal. When these had returned to normal, pedalling was resumed with the next heavier load and the above procedure was again followed. During the more protracted periods of recovery, after more difficult work, the subject was allowed to shift from the saddle to a high stool near the bicycle where he could sit more comfortably.

Before passing to a consideration of our data, it should be noted that a reduction in barometric pressure alone causes a rise in the percentage of carbon dioxide in the expired air. This effect is present both during rest and work and has frequently been recorded by students of Alpine physiology. According to our observations this barometric influence is exerted in equal degree both during rest and exercise.

Contrary to expectation we failed to find that a lowering of barometric pressure caused a definite shift in the position of the crest-load. Typical data for the experiments on three subjects are tabulated in table 1. The percentage output of carbon dioxide for H. M. at sea level was maximal (crest-load) and equal for two loads, 4,000 and 6,000 foot pounds, and was diminished with a load of 8,000 foot pounds. Briggs found that in most cases the expired carbon dioxide percentage curve gradually flattens as the crest-load is approached. Our experience conforms with his. For

TABLE 1

The elimination of carbon dioxide during work

LOAD IN FOOT-POUNDS	PERCENTAGE OUTPUT CO_2 IN EXPIRED AIR					THE OUTPUT OF CO_2 PER MINUTE IN CUBIC CENTIMETERS				
	Sea level	10,000 feet	15,000 feet	20,000 feet	25,000 feet	Sea level	10,000 feet	15,000 feet	20,000 feet	25,000 feet
					H. M.					
0	3.60	4.28	5.94	6.10	6.80	236	187	232	244	334
2,000	4.28	5.77	6.69	7.05	7.66	843	793	849	816	847
4,000	5.16	5.93	6.80	7.03		1.505	1.441	1.604	1.522	
6,000	5.16	5.89	6.48	7.19		1.948	1.909	1.853	1.805	
8,000	4.48					2.621				
					C. R. J.					
0	3.23	4.12	5.45	6.72	6.33	253	292	274	310	355
2,000	4.08	4.82	6.72	7.54	6.88	744	779	937	914	940
4,000	4.49	5.84	6.64	7.54	7.57	1.458	1.426	1.587	1.625	1.539
6,000	4.65	5.89	6.89	6.30		1.632	2.060	2.264	2.180	
8,000	4.50	4.94	5.94			2.533	2.728	2.858		
10,000	4.12	4.68				3.761	2.915			
					S. S. Y.					
0	3.01	4.06	4.53	5.09		224	212	213	254	
2,000	3.80	4.98	5.36	5.92		864	885	953	931	
4,000	4.06	5.10	5.78	6.05		1.489	1.687	1.633	1.488	
6,000	3.97	4.44				1.874	2.299			

H. M., therefore, the crest-load at sea level was somewhere between 4,000 and 6,000 foot pounds. At the simulated altitudes of 10,000 and 15,000 feet, it seemed to be 4,000 foot pounds; while at 20,000 feet, it was as definitely placed at 6,000 foot pounds. When H. M. was at a simulated altitude of 25,000 feet a complete series of loads was not carried, yet it is evident from the ascent of the curve, in that it is as steep as other curves and parallel to them, that 2,000 foot pounds was an easy normal-load and not a crest-load.

For C. R. J. the crest-load was clearly placed at 6,000 foot pounds at barometric pressures comparable to those of altitudes of sea level, 10,000 and 15,000 feet. At the simulated altitude of 20,000 feet it did not appear to be above 4,000 foot pounds; but at that of 25,000 feet, it certainly was not under 4,000 foot pounds and may have been as large as 6,000 foot pounds. In the experiments on S. S. Y. the crest-load was the same, 4,000 foot pounds, at all pressures—namely, those comparable to altitudes at sea level, 10,000, 15,000 and 20,000 feet.

In another subject, A. L. H., the crest-load was found to be 4,000 foot pounds at pressures comparable to altitudes of sea level, 10,000, 15,000

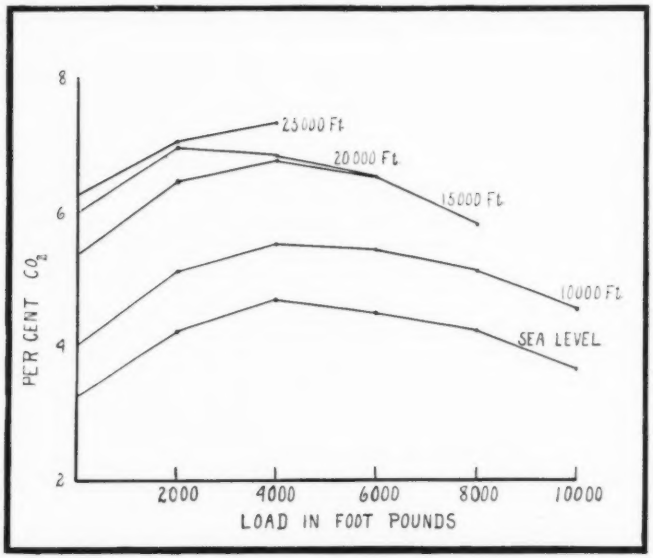

Fig. 1. Curves of the average per cent of exhaled carbon dioxide that indicate the position of the crest-load.

and 18,000 feet. For R. W. C. the crest-load was 6,000 foot pounds at pressures comparable to sea level and 10,000 feet, and 4,000 foot pounds at the simulated altitudes of 15,000, 20,000 and 25,000 feet.

We have prepared generalized curves of our data of the percentage output of carbon dioxide by averaging the results obtained from five subjects. The curves so established are given in figure 1. With the exception of the one at 20,000 feet, the curves are parallel and show that the maximum percentage output of carbon dioxide occurred with a load of 4,000 foot pounds at all barometric pressures down to 290 mm., which is comparable to an altitude of 25,000 feet.

An interpretation of these results must now be sought. If, as we have shown, the consumption of oxygen is reduced during work at a low barometric pressure, then the actual production of carbon dioxide must also fall off and, provided nothing occurs elsewhere to liberate some of the preformed carbon dioxide of the blood, then the percentage and volume output of this gas should also be decreased. According to the modern theory of muscle contraction lactic acid is set free in the process of contraction and is oxidized and reconverted to lactic acid precursor in the after period. If the lactic acid cannot be oxidized and reconverted to the normal extent because of lack of oxygen, then some of it should escape into the blood. A larger amount of lactic acid thus thrown into the blood plasma when work is done at a low barometric pressure would set free an unusually large amount of preformed carbon dioxide from the sodium bicarbonate of the blood plasma and this might account for the maintained sea level percentage of carbon dioxide elimination at the low barometric pressure.

It is evident from our data and from the above explanation that the percentage output of carbon dioxide, when work is done at low barometric pressures, is not a reliable index of normal-load, crest-load and over-load.

If it is the escape of lactic acid from the working muscles that accounts for the similar and parallel curves (fig. 1) of the percentage output of carbon dioxide when work is done at low barometric pressures, then the total volume output of carbon dioxide should be somewhat greater at low pressures than the extent of oxygen consumption would lead us to expect.

The volume of carbon dioxide eliminated during exercise. Under the conditions of our experiments at low barometric pressures, the volume of carbon dioxide eliminated can not be regarded as a reliable index of metabolism. During short exposures to a low barometric pressure, even when one is at rest, there occurs at first a blowing off of carbon dioxide due to the fact that the respiration is increased by the stimulating effect of a lack of oxygen on the respiratory center. This anoxemic blowing off of carbon dioxide is of variable duration. In some cases it is completed within from ten to twenty minutes, but in others hours are required (Schneider, Truesdell and Clarke, 1925). This process of blowing off carbon dioxide must be kept in mind as we continue our study of the output of carbon dioxide during exercise. It explains the high respiratory quotient which quite constantly occurs during the rest period that preceded the work of our experiments under a low barometric pressure.

We (Schneider and Clarke, 1925) have shown that lowering the barometric pressure 235 mm., equivalent to ascent to an altitude of 10,000 feet, at first, in most men, causes a reduction in the rate of oxidation in the muscles of the body both during rest and physical work. The decrease in the rate of oxidation during work holds a linear relationship to the

reduction in barometric pressure, being more pronounced the greater the reduction in pressure. This decrease in oxidation, or oxygen consumption, varies with the load of work. With a load of 4,000 foot pounds, it has ranged for our cases from ten to twenty per cent at a barometric pressure of 375 mm., approximately equivalent to an altitude of 20,000 feet. If it were not for the blowing off of carbon dioxide that is caused by the stimulating effect of oxygen want, a similar decrease in the volume output of carbon dioxide should likewise show when work is done under a low barometric pressure. We find, however, that this is not the case. Instead the quantitative elimination of carbon dioxide is practically the same at all pressures for any load, or at times is even greater at low pressures than at sea level pressure. The data for three subjects are given in table 1. The data for each are gathered from a number of experiments on widely separated days and, therefore, lack the uniformity of step experiments conducted on a single day. In table 3 of our first paper of this series (Schneider and Clarke, 1925) are given the results of step experiments with three subjects. Of these R. W. C., with a minute-load of 2,000 foot pounds, had an output of 691 cc. of carbon dioxide at a barometric pressure of 776 mm.; 703 cc., at 548 mm.; 697 cc., at 463 mm.; and 705 cc., at 391 mm. With the same load E. W. G. eliminated 765 cc. of carbon dioxide at 760 mm.; 743 cc., at 532 mm.; 782 cc., at 447 mm.; and 898 cc., at 375 mm. H. M., however, unlike the results shown in table 1 of this paper, gave a moderate decrease in the output of carbon dioxide at the lowest pressures with loads of 2,000 and 6,000 foot pounds, but the decrease did not correspond to the reduction in the consumption of oxygen.

The equality of output of carbon dioxide is present throughout the whole of a work period. The entire respiratory exchange has been determined during a work period of eight minutes and during an after-period of 22 minutes. The data for a set of three experiments on R. W. C. at barometric pressures of 764, 447 and 415 mm. with a load of 4,000 foot pounds are given in table 4 of our first paper (Schneider and Clarke, 1925). During a work period the elimination of carbon dioxide rises rapidly for the first two minutes and in the third or fourth minute reaches a more or less steady state, as is also the case with the intake of oxygen. The intake of oxygen in the three experiments on R. W. C. was materially decreased by the reduction in barometric pressure; it was for the entire period of eight minutes 11,023 cc. at 764 mm., 10,088 cc. at 447 mm., and 9,024 cc. at 415 mm. The carbon dioxide output, however, was practically equal during the three work periods; it was 9,958 cc. at 764 mm., 10,057 cc. at 447 mm., and 9,987 cc. at 415 mm.

In a similar series of three experiments on E. W. G. the oxygen consumption in the eight minutes of work was 10,599 cc. at 756 mm., 8,305 cc. at 457 mm., and 8,501 at 415 mm. Although the intake of oxygen was less

at the low pressures than at sea level, yet the output of carbon dioxide was equal at the three pressures; it was 10,640 cc. at 756 mm., 10,392 cc. at 457 mm., and 10,618 cc. at 415 mm. The data for a pair of experiments on H. M. are given in table 5 of our first paper (Schneider and Clarke 1925) in which, under the low pressure, the output of carbon dioxide fell off. He eliminated, in eight minutes of work, 11,469 cc. at 760 mm. and 10,677 cc. at 415 mm. However, in another work period at 455 mm. he eliminated 12,132 cc.

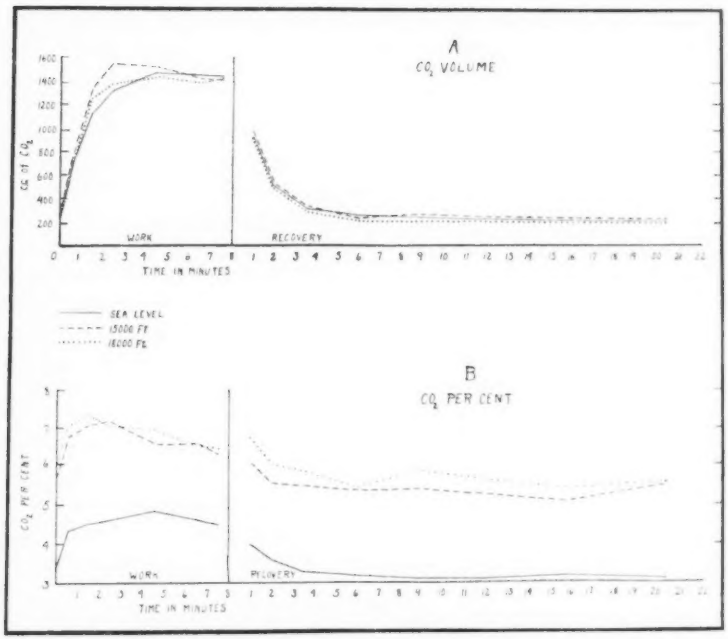

Fig. 2. The elimination of carbon dioxide during and immediately following exertion at 3 barometric pressures.

Composite curves of the output of carbon dioxide during exercise have been prepared by taking the averages from a series of experiments on four subjects at pressures comparable to altitudes of sea level, 15,000 and 18,000 feet. These have been plotted in A of figure 2. While the three curves are not identical, they so nearly coincide that they give convincing evidence of the equality of carbon dioxide elimination at the several barometric pressures here considered.

The per cent of carbon dioxide that is in the expired air minute by

minute during the eight minutes of work is compared in B of figure 2 for three barometric pressures. The data there plotted are the averages from experiments on the same four subjects used in the study of the volume output of carbon dioxide. At sea level, when a constant load of work is carried, the percentage output of carbon dioxide ordinarily reaches a steady state by the third minute. However, when work is done under a low barometric pressure, a steady state does not occur. Instead, after the rise in the percentage output of carbon dioxide of the first three minutes, there occurs a gradual moderate drop through the remaining minutes of work. But in no case does the drop bring the percent of exhaled carbon dioxide back to the pre-exercise value.

The respiratory quotient. The respiratory quotient is used at times to give evidence regarding the fluctuations of lactic acid in the body. Under the conditions with which we worked, it is to be expected that the respiratory quotient is liable to show temporary variations which are dependent on other factors than alterations in the proportions in which carbohydrate and fat are metabolised. In prolonged steady exercise, the respiratory quotient often varies with the diet. However, in severe exercise, or when work is done with insufficient oxygen, it is believed that a considerable amount of lactic acid escapes from the muscles. Should this occur it will lead to an expulsion of carbon dioxide from the preformed carbon dioxide held in combination in the blood. This would result in an increased elimination of this gas from the body without a corresponding increase in oxygen consumption or any necessary change in the character of the metabolism. The modern theory of muscular contraction led us to expect that work done under a low barometric pressure would very materially increase the lactic acid content of the blood. We have, however, shown in our first paper of this series that the "oxygen debt" and "oxygen lag," two conditions that give an indication of the amount of lactic acid that has accumulated in the blood and muscles, were not greater when work was done at low barometric pressures than when the same work was done at sea level pressure. It is of interest, therefore, to examine our data for the respiratory quotient to find what evidence they contain.

A typical curve which shows changes in the respiratory quotient for exercise at sea level barometric pressure, is given in figure 3. The curve was prepared by taking the averages of seven experiments on four subjects. During the first minute of work the respiratory quotient usually was slightly lowered, it fell in six of our experiments and rose in the other. After this it rose, reaching a maximum during the fourth or fifth minute, following which it slowly diminished as work continued. In one experiment it remained constant after the maximum was attained.

The effect of low barometric pressures on the exercise respiratory quotient is shown in the curves for 15,000 and 18,000 feet in figure 3. The

respiratory quotient always rose during the first minute in our low pressure experiments, continued to rise rapidly during the next two minutes and then diminished. The most striking difference, with the possible exception of the rapid rise in the first minute, is the fact that the respiratory quotient remains above unity when work is done under the lowest pressures.

An interpretation of these differences of the respiratory quotient, as they occur during work at sea level pressure and at low barometric pressures, based on the lactic acid theory alone, does not seem adequate. In order to account for the immediate rise in the respiratory quotient that occurs under a low barometric pressure, the muscles would have to discharge a considerable amount of acid at the very beginning of exercise. Theoretically lactic acid should not be discharged into the blood stream

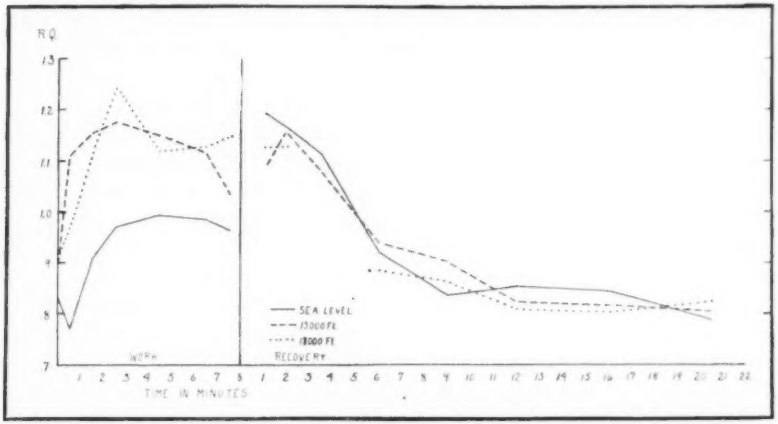

Fig. 3. The respiratory quotient during and following work at 3 barometric pressures.

in significant quantities, or at all, so long as a load is a normal-load. A load of 4,000 foot pounds for our subjects was either a normal-load or at most a crest-load. Had it been an over-load a discharge of acid into the blood would have occurred. Campbell, Douglas and Hobson (1920) suggest that, with moderate work, want of oxygen might be responsible for changes in respiration that alter the respiratory quotient. There is plenty of evidence to prove that the respiratory center is very responsive to changes in the oxygen supply and that it is stimulated by a lack of oxygen. Want of oxygen would adequately account for the rise in the respiratory quotient that occurred during the first minute of exercise in our experiments under the low barometric pressures. The effect of oxygen want on the respiratory center superimposed on the effects of an accumulation of

lactic acid during the next minutes of work might account for the fact that the quotient so constantly remains above unity at low barometric pressures. But it may be that anoxemia alone accounts for the higher value.

The interplay of the several respiratory features that we have examined should be borne in mind in the consideration of the respiratory quotient. During exercise, under a low barometric pressure, the absorption of oxygen is decidedly less than when the same amount of exercise is taken at sea level pressure. After the third or fourth minute the usage of oxygen becomes practically constant. The minute-volume of breathing augments rapidly during the first three minutes and thereafter tends to remain constant when exercise is taken at sea level, while at a low barometric pressure the minute volume augments more rapidly during the first minutes than it does at sea level and thereafter usually continues to augment steadily but slowly. At the same time the percentage output of carbon dioxide rises rapidly during the first two minutes of exercise, then maintains a fairly constant level at the sea level barometric pressure, but shows a steady slow fall at low barometric pressures. The total output of carbon dioxide at all pressures rises rapidly during the first three minutes and then remains fairly constant through the remainder of the work period. The fall in the percentage output of carbon dioxide at low barometric pressures proves that during the latter part of the work period less carbon dioxide is moved per liter of ventilation and, therefore, that the production of carbon dioxide is not equal to the elimination. There is, therefore, a real blowing off of carbon dioxide which is either due to the effect of anoxemia on the respiratory center or to the stimulating action on the center of an increase in the acid content of the blood brought about by lactic acid contributed by the active muscles.

The changes during the period of recovery throw some light on the question as to which of the above two possibilities may cause the observed changes in the respiratory quotient. The recovery changes of the respiratory quotient are given graphically in figure 3 for experiments conducted at barometric pressures comparable to those of the altitudes of sea level, 15,000 and 18,000 feet. The data are the averages of all experiments at the several pressures. At each pressure the respiratory quotient usually remained above unity for from three to four minutes after the close of the period of work. During this interval the respiratory quotient for sea level was slightly above those obtained at the low barometric pressures but the differences were not of sufficient magnitude to be significant. During the following portion of the 22 minutes of the recovery period that was studied the respiratory quotient fell at approximately the same rate and in equal amounts at all pressures. According to the modern theory (Hill, Long and Lupton, 1923), during the initial recovery period, when

the respiratory quotient is above unity, some carbon dioxide is turned out from the blood bicarbonates by lactic acid that still continues to escape from the muscles. Our curves of the averages and of individual cases all indicate that the amount of acid eliminated by the muscles in this period was, on the whole, slightly greater after exercise at sea level than at the simulated altitudes of 15,000 and 18,000 feet. Hill, Long and Lupton (1923), in a study of isolated muscle, have shown there is strong evidence that the respiratory quotient of the recovery oxidations is unity, lactic acid itself being oxidized. So long then as lactic acid itself is being oxidized in the muscle in the reconversion process of the period of recovery, the use of oxygen and output of carbon dioxide should be equal. However, when the body begins to oxidize the lactic acid that has escaped into the blood, then some of the carbon dioxide that is being formed, instead of being immediately eliminated by the lungs, will be used to combine with the base that is liberated by the oxidative removal of the acid. In so far as this occurs the respiratory quotient will fall below unity. When this happens the body passes into what is known as the second phase of the recovery process, the retention phase, and the carbon dioxide held back may be used as a measure of the lactic acid removed from the blood. Our respiratory quotient curves for the second phase of the recovery period are so nearly alike for the three barometric pressures studied that we may assume that the amount of lactic acid in blood is no greater when a particular load of work is done under a low barometric pressure than when that load is carried at sea level pressure.

Our experiments support the theory that the equality of output of carbon dioxide during exercise at low and high barometric pressures and the higher respiratory quotient during exercise at a low barometric pressure are due to the direct effect of lack of oxygen on the respiratory center rather than to an increase of the lactic acid in the blood.

The above conclusion is supported by other features of the recovery period: 1. The average amount of oxygen used by our subjects in 22 minutes after carrying a load of 4,000 foot pounds for eight minutes was no greater when work was done under a low pressure than when the same amount of work was done at sea level pressure. The average amount of oxygen used in the recovery period was 7,067 cc. at sea level; 7,097 cc. at the simulated altitude of 15,000 feet; and 6,579 cc., which was slightly under the normal usage, at the simulated altitude of 18,000 feet. An excess of oxygen should have been used after work at low barometric pressures had the acid been the regulating factor, in that it would have accumulated during work and would have to be oxidized and reconverted to glycogen afterward. 2. The average total amount of carbon dioxide eliminated in the recovery period at the three pressures proportionately corresponded rather closely to the oxygen usage and seems, therefore,

to have been determined more by the rate of metabolism than amount of acid change. The amount of carbon dioxide eliminated in 22 minutes was 6,573 cc. at sea level, 6645 cc. at the simulated altitude of 15,000 feet, and 6,022 cc. at the simulated altitude of 18,000 feet. The curves for the minute by minute elimination of carbon dioxide very nearly coincide (see A, fig. 2). 3. The minute-volume of breathing during the recovery period likewise returns to normal so quickly and in so nearly the same way at all pressures as to render doubtful the explanation of the respiratory change by the acid theory. In table 2 the average minute-volumes are given for the recovery period of our experiments conducted under three different barometric pressures. For the first two minutes after work, the volume of air breathed was greater at the low barometric pressures than at that of sea level. During the third or fourth minute the breathing became about equal at each of the simulated altitudes and thereafter was gradually reduced in equal amounts at the three pressures. Curves representing the post-exercise volume of breathing practically coincide.

TABLE 2

Average minute-volume of breathing during a recovery period of 22 minutes, in liters

BAROMETER	1	2	3, 4	5, 6, 7	8, 9, 10	11, 12, 13	15, 16, 17	19, 20, 21, 22
mm.								
760	25.5	15.7	11.8	10.6	9.1	8.6	8.1	7.6
445	31.0	18.4	12.5	10.3	9.4	8.8	8.5	7.9
415	29.7	18.1	11.2	9.5	8.8	8.8	8.6	8.0

The above facts strengthen the theory that the exercise respiratory and gaseous exchange differences, brought about by a reduction of barometric pressure, are almost entirely a direct action of oxygen deficiency on the respiratory center.

SUMMARY

A reduction of barometric pressure increases the carbon dioxide percentage of the exhaled air of rest and physical exertion.

Ordinarily the exhaled percentage of carbon dioxide first rises and then falls as the load of work is augmented. At reduced barometric pressures added increments of work cause a rise and fall similar to that obtained at sea level, but the curves representing the changes start at higher percentages at low atmospheric pressures. The curves for various pressures are roughly parallel.

During the early part of a stay at a reduced pressure the minute volume output of carbon dioxide is not a measure of gaseous metabolism even during rest because some of the preformed carbon dioxide is at first blown

off as a result of the stimulating action of oxygen-want on the respiratory center.

With any given load of work the volume of carbon dioxide eliminated remains about the same at all barometric pressures that we have studied, down to 290 mm., which is approximately equivalent to an altitude of 25,000 feet.

While the volume of carbon dioxide that is eliminated during physical exertion is not changed by a reduction of barometric pressure, the consumption of oxygen is decidedly lessened. This is interpreted to mean that an unusual amount of preformed carbon dioxide is blown off from the blood when work is done at low atmospheric pressures. It is this blowing off of preformed carbon dioxide that keeps the minute output at a low pressure equal to that at sea level pressure.

Evidence obtained from the respiratory quotient indicates that the blowing off of preformed carbon dioxide at low barometric pressures during physical exertion is chiefly the result of a direct action of want of oxygen on the respiratory center and not to an increased outpouring of lactic acid from the muscles to the blood.

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STUDIES ON THE OCCURRENCE OF EXTENSOR RIGIDITY IN MAMMALS AS A RESULT OF CORTICAL INJURY

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Since Sherrington (1897) first described the phenomenon of "decerebrate rigidity" many investigators have sought to determine the seat of origin of this condition. The work of Sherrington, Weed (1914), Bazett and Penfield (1922), Magnus (1924) and Rademaker (1926) indicates that the main centres involved in the production of the decerebrate rigidity lie in the brain-stem and probably extend as far caudalward as Deiter's nucleus. Decerebrate rigidity is probably a release phenomenon which demonstrates itself following the removal of the higher centres in the brain-stem and cerebral hemispheres. The exact portions of the central nervous system, release from which results in the increased extensor tonus, are not as yet definitely known.

Munk (1895) stated that, in apes, "contracture" of the limb muscles not infrequently follows extirpation of the corresponding motor areas of the cerebral cortex. On the other hand he reported that extirpation of the motor cortex of rabbits, cats and dogs fails to produce contracture in the corresponding limbs. King (1927) observed that, in cats, extensive removal of the electrically excitable cortex alone, is never followed by hypertonus even immediately after the operation. He further observed that removal of the cortical areas, which he designates "frontal lobe" results in extensor rigidity in the contralateral limbs. He concluded that the frontal lobe area and the area designated "A" comprise a cortical centre whose integrity is essential for normal postural adjustments.

Horsley (1906) pointed out the identity of decerebrate rigidity in animals with the rigidity of the leg in hemiplegia in man. Walshe (1914), (1919) and (1923) from a study of patients with spastic paralysis, spinal lesions and cerebral lesions concluded that the extended type of spastic paralysis in the legs is identical with the decerebrate rigidity observed by Sherrington in animals and possessed similarly a mesencephalo-spinal reflex arc. He further stated that an uncomplicated pyramidal lesion always gives rise to the extended form of spastic paralysis in the legs. Brain (1927) showed that the flexor spasticity in the arms in hemiplegia can be transformed into one of extension if the patient assumes the quadrupedal attitude. He

considers that the flexor posture may be considered as a modification of the extensor evoked by the assumption of the erect attitude. The observations of Richter and Bartemeier (1926) that decerebrate rigidity in the sloth manifests itself by flexor spasms in the limbs, claws and trunk and in reflex hanging, in keeping with its normal posture, are of considerable interest in relation to the flexor spasticity of hemiplegia.

In view of the conclusions of Walshe, Brain and others that the spasticity of hemiplegia in man is due to pyramidal tract lesions and that contracture occurs in the limbs of apes after the removal of the corresponding motor cortex (Munk) I considered that, in the lower animals such as the cat, dog, rabbit and guinea pig extensor rigidity might be produced by injury of the pyramidal tract system at its origin, i.e., by injury to the motor cortex. With this idea in view the present series of experiments was undertaken.

METHODS. In the present work rabbits, cats, dogs and guinea pigs were used; the procedure adopted was the same for each type of animal, observations being made in both acute and chronic preparations.

Acute experiments. The animals were anesthetised with ether, the skull trephined and the portion of the cortex, which on stimulation caused muscular movements in the limbs, exposed. In cats the approach was made through the frontal sinus as the sigmoid gyrus lies far forward in the cerebral hemispheres. The cortex was kept warm with small pads of absorbent cotton soaked in warm Ringer-Locke solution. The anesthetic was now diminished and the motor cortical areas explored with either bipolar or unipolar electrodes. For stimulation either a Cambridge or Kershaw coil was used, with a current of 0.4 ampere in the primary the secondary distance being 100 to 120 mm. The excitable cortex for the limbs was identified by the appearance of movements in the contralateral fore- and hind-legs during the stimulation. In rabbits and guinea pigs the fore-leg areas alone were studied as it is difficult to obtain movements from the hind-legs as a result of cortical stimulation. In cats and dogs the effects of removal of both fore- and hind-leg areas were studied. The extent of the motor cortex for the limbs having been determined the next step was to ablate this area either with a knife or with an electric cautery. The anesthetic was now removed and the animal allowed to recover. Observations were made during the recovery period and also during the period following when the effects of the anesthetic had entirely disappeared.

Chronic experiments. In these experiments the operation was done aseptically. The procedure adopted was identical with that already described for the acute experiments. Observations were made for days or weeks following the operation.

Control experiments. Control experiments were done on all the types of animals studied. In these experiments the skull was trephined and por-

tions of the cerebral cortex remote from the excitable cortex for the limbs were removed. In cats special attention was given to the area frontalis and the effects of stimulation and removal of portions of this area were noted.

OBSERVATIONS. *Rabbits. Acute experiments.* The cortical fore-leg area in the rabbit is readily excitable with both bipolar and unipolar stimulation. The movements observed in the majority of rabbits are flexion at the elbow and shoulder. In some, however, flexion at the wrist is observed and also rhythmical flexion and extension of the leg. Following unilateral removal of the fore-leg area, either with a knife or cautery, definite symptoms develop almost immediately, in the contralateral fore-legs. With the animal in the prone position, the line of the mouth making an angle of 45° above the horizontal (position of minimal tonus) (Magnus, 1924), there is a definite extensor rigidity in the contralateral fore-leg (fig. 1). The leg extends, not infrequently, of its own accord but if it does not so appear, gentle pressure on the plantar surface of the paw invariably results in its appearance. Similar extensor rigidity is not observed in the ipsilateral fore-leg. These symptoms are present 3 to 5 minutes after the ablation.

After the animal has recovered from the effects of the anesthesia the above-mentioned symptoms are still present. If the animal is placed in

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Fig. 1. Rabbit, following unilateral extirpation of the motor cortex for the fore-leg, showing extensor rigidity in the contralateral fore-leg.

Fig. 2. Same rabbit as in figure 1, in the dorsal decubitus showing extensor rigidity in the contralateral fore-leg.

Fig. 3. Same rabbit as in figure 1, showing paralysis in the affected fore-leg.

Fig. 4. Brain of rabbit shown in figures 1, 2 and 3, two weeks after the operation, showing the cortical lesion.

Fig. 5. Guinea pig, following unilateral extirpation of the cortical fore-leg area, in the dorsal decubitus showing extensor rigidity in the corresponding fore-leg.

Fig. 6. Dog, following unilateral extirpation of the motor cortical areas for fore- and hind-legs, in the dorsal decubitus showing extensor rigidity in the contralateral fore- and hind-legs.

Fig. 7. Same dog as shown in figure 6, showing paralysis in the contralateral fore- and hind-legs.

Fig. 8. Brain of dog shown in figures 6 and 7 two weeks after the operation showing cortical lesion. The lesion is outlined with bristles.

Fig. 9. Dog, following extirpation of the motor cortical areas for the fore-leg, showing rigidity and paralysis in the contralateral fore-leg.

Fig. 10. Cat, following unilateral extirpation of the motor cortex for fore- and hind-legs, showing paralysis in the corresponding legs.

Fig. 11. Same cat as shown in figure 10, in the dorsal decubitus showing extensor rigidity in the affected legs.

Fig. 12. Cat shown in figure 11, suspended, showing rigidity in the affected legs.

Fig. 13. Brain of cat, two weeks after operation, showing cortical lesion. The lesion is outlined with bristles.

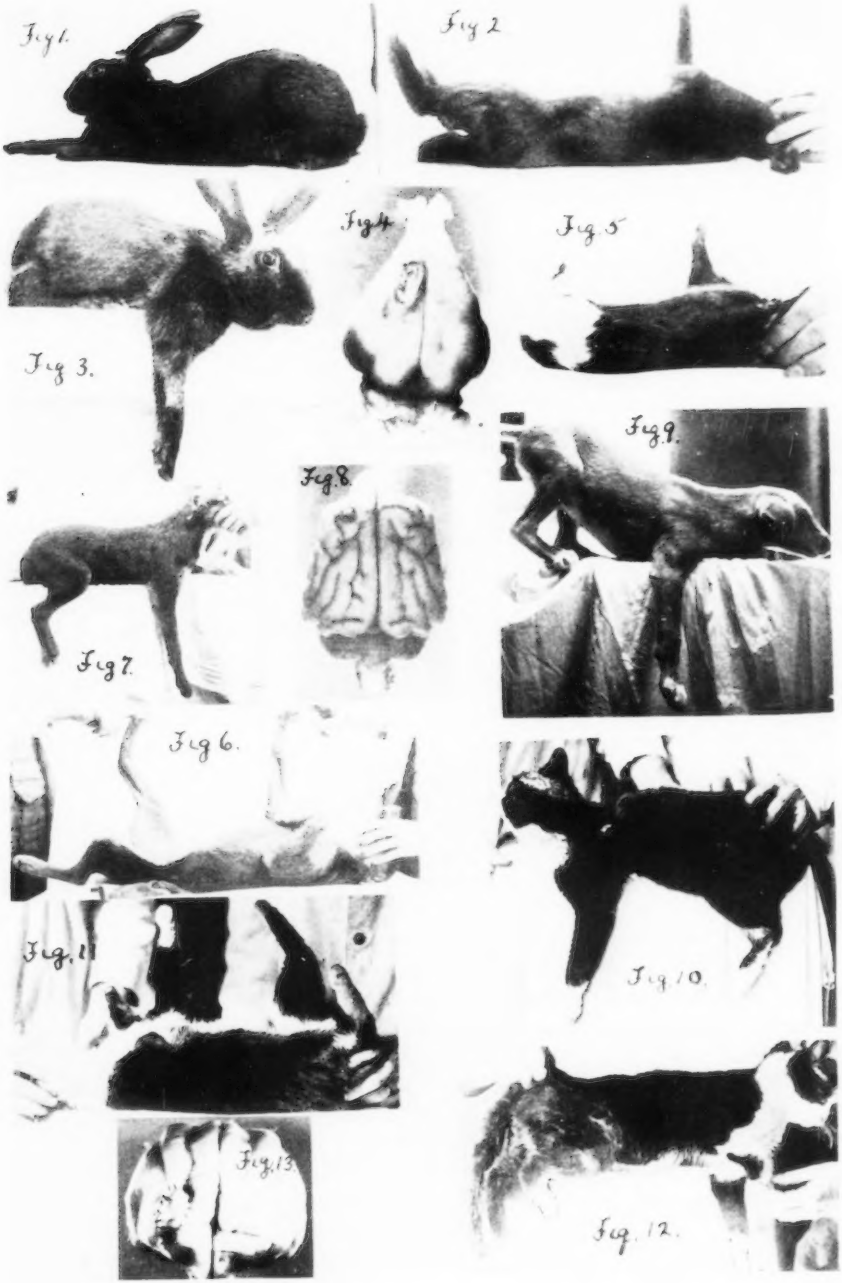
Fig 1.
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the dorsal decubitus (Magnus, 1924) the extensor rigidity in the fore-legs opposite to the cortical lesion becomes more intense (fig. 2). It resembles the rigidity described by Sherrington in the decerebrate animals and is modified by labyrinthine and neck reflexes in a manner similar to decerebrate rigidity (Magnus, 1924). The affected leg also shows marked motor paralysis. If the animal is supported on a table with the affected limb hanging over the edge (fig. 3), the animal is unable to withdraw the pendent leg. This is not the case with the ipsilateral fore-leg. When it is placed in a similar position it is rapidly withdrawn. Furthermore if the affected leg is placed in any abnormal position it remains where placed until the animal attempts to hop when all the legs are brought into action. While hopping or walking there is a definite incoördination in the contralateral fore-leg the foot being dragged along the floor the animal frequently coming to rest with the foot flexed at the wrist.

Rabbits. Chronic experiments. The observations, already described for the acute experiments, are duplicated in the chronic experiments during the first few days following the operation. After about six days have passed the symptoms become less marked. The paralysis gradually becomes less and disappears after ten or twelve days. The rigidity also becomes less marked and after about two weeks it can only be demonstrated with the animal in the dorsal decubitus. It is noteworthy that, in the majority of the experiments, rigidity can be demonstrated for some time after the paralysis has disappeared.

After the effects of the ablation had disappeared the animals were killed and the brain and spinal cord preserved in formalin for further study. At autopsy the brains of these animals showed clear cut cortical lesions with little or no hernia (fig. 4).

Guinea pigs. Acute and chronic experiments. Observations on guinea pigs were similar to those described for rabbits. Ablation of the motor cortex, which on stimulation yields flexion in the contralateral fore-leg, results in a marked extensor rigidity in the corresponding limb. When the animal is held in the maximal position (Magnus) the rigidity is more pronounced (fig. 5) although it can also be demonstrated when the animal is held in the minimal position (Magnus).

Dogs. Acute experiments. Ablation of the excitable area in the right hemisphere which on stimulation yields flexion in the left fore-leg, results in extreme extensor rigidity in that leg within two minutes after the removal; the animal being in the prone position with the head supported so that the line of the mouth makes an angle of 45° below the horizontal. The right fore-leg is flaccid as determined by pressure applied to the sole of the foot. Ten minutes after the ablation the rigidity in the affected leg is intense at the shoulder and elbow but very slight at the wrist. As the animal recovers from the effects of the anesthetic, extensor rigidity is

noticed in the ipsilateral fore-leg and also in the hind-legs but it is not so intense nor does it respond to stimuli as does the extensor rigidity in the contralateral fore-leg. After the effects of the anesthetic have passed off the animals show symptoms of profound shock and the extensor rigidity in all the legs disappears and does not reappear until several hours have elapsed.

When the motor areas, which on stimulation gave muscular movements in the contralateral fore- and hind-legs, are ablated extensor rigidity develops almost immediately in the corresponding legs. The character of the extensor tonus in the affected fore-leg is identical with that already described when the fore-leg area alone is removed. In the affected hind-leg it is present in the hip, knee and ankle. As in the experiments described above, the rigidity disappears temporarily after the effects of the anesthetic have passed off.

Dogs. Chronic experiments. In these experiments, the initial symptoms which developed immediately after the ablation of the leg areas, were identical with those described for the acute experiments. Twenty-four hours after the operation, the extensor rigidity in the legs contralateral to the lesion can be readily demonstrated. If the lesion involves both fore- and hind-leg areas a marked extensor rigidity is evident in the corresponding legs, the animal being held in the dorsal decubitus (fig. 6). The rigidity is also present when the animal is held in the ventral decubitus but it is not so intense. In both positions the intensity of the rigidity is greater in the fore-legs than it is in the hind-legs. In these animals paralysis is present in the affected fore- and hind-legs as shown by the tests already described (fig. 7). Coördination in the affected limbs, while walking, is poor. The legs support the body and can perform the locomotory movements but the paws drag along the floor and if the animal is interfered with to a slight degree it may lose its balance sinking to the floor with the paralyzed limbs sprawled out in abnormal positions.

The paralysis and rigidity can be shown to be present with undiminished intensity for four or five days. Following this period the rigidity gradually disappears, the rate of disappearance being more rapid in the hind-legs. It can however be demonstrated in the dorsal decubitus two weeks or more after the ablation. The paralysis disappears somewhat earlier than the rigidity and after about ten or twelve days it cannot be demonstrated by the tests used. In every case the paralysis and rigidity disappear earlier in the hind-legs than they do in the fore-legs. Two weeks after the operation the animals were killed and the central nervous system preserved for histological study. Figure 8 shows the brain of a dog two weeks after the ablation of the fore- and hind-leg areas in the left hemisphere.

If the fore-leg area alone is removed rigidity and loss of voluntary

control is evident in the contralateral fore-leg. The effects of the ablation on the fore-leg are similar to and run a course identical with that already described for the fore-leg when both fore- and hind-leg areas were removed. Figure 9 shows a dog following the removal of the fore-leg area in the left hemisphere. The contralateral fore-leg shows a marked extensor rigidity and complete loss of voluntary control.

Cats. Acute experiments. Observations were made following unilateral ablation of the excitable cortex for fore-leg and hind-leg separately and also following ablation of both areas together. The effects of stimulation and removal of the area frontalis were also noted.

Following the removal of a portion of the sygmoid gyrus, which on stimulation gave muscular movements in the contralateral fore-leg, a marked extensor rigidity develops almost immediately in the corresponding fore-leg. During the period in which the anmal is recovering from the anesthetic there is an extensor rigidity in all the legs but the intensity is greater in the contralateral fore-leg. After the effects of the anesthetic have completely passed off the rigidity is still present in the contralateral fore-leg in both the dorsal and ventral decubitus but is absent in the other legs. Loss of voluntary control in the contralateral fore-leg is evident as shown by the tests previously described.

Removal of a portion of the posterior sigmoid gyrus, which on stimulation caused muscular movements in the contralateral hind-leg, results in a marked extensor rigidity in the corresponding limb which persists after the effects of the anesthetic have completely disappeared. Paralysis in the affected hind-leg is profound.

Removal of both fore- and hind-leg areas results in a marked extensor rigidity in the corresponding limbs. The rigidity persists with varying intensity regardless of the position of the animal. It is always more marked in the dorsal decubitus and invariably it is more intense in the fore-leg than it is in the hind-leg. Following this ablation the paralysis in the contralateral legs is marked being more profound in the fore-legs.

Electrical stimulation of the area frontalis failed to elicit muscular movements in all experiments. In several experiments, however, we noted muscular movements in the neck and face on the contralateral side. These movements caused lateral flexion of the head to such an extent, in one animal, that the head was withdrawn from the ether cone. Removal of portions of the area frontalis, in acute experiments, failed to produce any extensor rigidity in any of the limbs or any demonstrable rigidity in the neck muscles.

Cats. Chronic experiments. The immediate effects of removal of the various portions of the excitable cortex for the limbs, under aseptic conditions, were identical with those already described for acute experiments. If both fore- and hind-leg areas are removed very definite symptoms are

evident twenty-four hours after the operation (fig. 13). The contralateral limbs show a profound paralysis the animal resting with the legs in abnormal positions, no effort being made to withdraw them (fig. 10). The animals are able to walk but in doing so the coördination in the affected limbs is poor, the paws drag along the floor and not infrequently the cat loses its balance sinking to the floor with the affected legs sprawled out in abnormal positions where they remain.

If the animals are placed in the dorsal or ventral decubitus marked extensor rigidity is observed in the contralateral fore- and hind-limbs (figs. 11 and 12). Gentle pressure applied to the feet results in a marked increase in the intensity of the rigidity. In all the experiments the extensor rigidity is much more intense in the fore-legs than it is in the hind-legs as shown by the pressure required to flex the legs and is modified by neck and labyrinthine reflexes in a manner identical with that observed in decerebrate animals (Magnus, 1924).

The conditions just described last for four or five days following which period the symptoms gradually disappear. The paralysis, which is always more marked in the fore-legs, gradually becomes less prominent as shown by the ability of the animal to use its limbs in a more normal manner. After about ten days there is no sign of paralysis in the hind-legs but it requires about twelve to fourteen days for its complete disappearance in the fore-legs. The extensor rigidity persists for a longer period following the operation than the paralysis and can be demonstrated in both fore- and hind-legs two weeks or more after the operation. It is always more marked in the fore-legs and persists longer in them than in the hind-legs.

If the fore-leg area alone is removed the symptoms of rigidity and paralysis are confined to the contralateral fore-leg. Their duration and quality are the same as that described for the fore-leg when both fore- and hind-leg areas are removed simultaneously. When the hind-leg area alone is removed the contralateral hind-leg alone is affected. The rigidity in the hind-leg is less intense than that which occurs in the fore-leg and the paralysis is less profound. Both paralysis and rigidity disappear earlier from the hind-leg than they do from the fore-leg.

Removal of large portions of the area frontalis does not produce extensor rigidity nor paralysis in the contralateral or ipsilateral limbs. The animals in these experiments, following the effects of the anesthetic, react in a manner similar to normal animals. At no time during the two weeks following the operation is there any indication of the symptoms, as described above, which result from the removal of the fore- and hind-leg areas. In some of these animals there is evident a stiffening of the neck muscles in response to certain stimuli. If the animal, while sitting quietly, be suddenly seized by the skin on the back of the neck the neck muscles become rigid and the head is drawn backwards. It is noteworthy

that in some of these animals, stimulation of the area frontalis caused contraction of the neck muscles on the contralateral side.

Control experiments. Rabbits, cats, dogs and guinea pigs. When inexcitable portions of the cortex, remote from the areas which on stimulation give movements in the contralateral limbs, are removed none of the symptoms described above for the various individual types are observed. The animals in the chronic experiments make a very rapid recovery and react in a manner similar to normal animals immediately after the effects of the operation have passed off.

Discussion. The observations above reported indicate that extirpation of the motor cortical areas for fore-limb or hind-limb results in definite extensor rigidity in the corresponding limb. Also when both fore- and hind-leg areas are removed simultaneously the extensor rigidity appears in the contralateral fore- and hind-limbs. Following the operation the extensor rigidity can be demonstrated with gradually diminishing intensity for two weeks or more in cats and dogs and for eight or twelve days in rabbits. After such periods elapse the animals appear normal. When both fore- and hind-leg areas are removed in the cat and dog the rigidity in the fore-leg is more intense than in the hind-leg and persists for a longer period.

Following the extirpations of these motor areas a profound paralysis is observed in the affected limbs. In the cat and dog the paralysis persists for about twelve days following the operation and for a somewhat shorter period in the rabbit. In every case the rigidity persists for a longer period than the paralysis. In the cat and dog, when both fore- and hind-leg areas are removed, the paralysis persists in the fore-leg for a longer period than it does in the hind-leg. In the present series of experiments on cats, extensor rigidity and paralysis failed to appear in the contralateral legs following ablation of portions of the area frontalis.

The extensor rigidity described in the present work resembles decerebrate rigidity in the manner in which it is modified by mechanical stimuli and also by neck and labyrinthine reflexes as described by Magnus and deKleijn (1912). It differs from true decerebrate rigidity in that it does not persist with undiminished intensity for long periods and can only be demonstrated when the animals are quiescent. One would not expect to obtain the intense extensor rigidity of the decerebrate animal in a preparation deprived only of the motor cortex. In these animals the reflex mechanisms in the brain-stem and spinal cord which function in the coarse coördinated movements are intact and able to carry on their normal functions. The author (1924) has shown that coördinated movements of progression are able to break through a marked extensor rigidity and are executed in a normal fashion. In the animals of the present work the nervous mechanism which functions in the coördination of the locomotory

movements is intact and when the animals commence walking the rigidity disappears. During the process of locomotion the paralytic symptoms already described are readily observed.

The observation on rigidity as described above are in accordance with those of certain other workers. Munk (1895) reported that, in apes, ablation of the motor cortex results in "contracture" in the muscles of the corresponding limbs. Olmsted and Logan (1925) observed that in cats, removal of the entire area about the cruciate sulcus results in extensor rigidity in the contralateral limbs. In this respect my results are in partial agreement. They further reported, however, that the degree of extensor rigidity depends on the completeness of the destruction of the area frontalis. In the present work no rigidity was present in the contralateral limbs of cats following the ablation of the area frontalis. In this respect the present results differ from those of Olmsted and Logan and also from those of King (1927) and Langworthy (1928) who are of the opinion that extirpation of the area frontalis in cats results in extensor rigidity in the contralateral limbs while extirpation of the motor cortical areas fails to produce the rigidity. The observations of Walshe on patients with hemiplegia, spinal lesions and cerebral lesions are of interest in view of the observations reported in this paper. He is of the opinion that the extended type of spastic paralysis is identical with the decerebrate rigidity as described by Sherrington for animals. He further contends that in an uncomplicated pyramidal lesion always results in the extended type of spastic paralysis.

That decerebrate rigidity is not due entirely to the release from cerebral control is evident from the observations of several workers. Rademaker (1926) is of the opinion that, in rabbits, decerebrate rigidity only occurs when the plane of transection passes through the red nucleus or fibre tracts leading from it. He showed that in mid-brain rabbits, which showed no rigidity, sectioning of the decussation of Forel results in an extreme extensor rigidity. The author (1924) has shown that decerebrate rigidity is present in mid-brain rabbits. F. R. Miller and the author working in this laboratory on mid-brain rabbits, in which, contrary to Rademaker's opinion, moderate but definite extensor rigidity was observed, noted that when the decussation of Forel is cut by a medial longitudinal incision the rigidity becomes much more intense. Histological examination of the brain-stems of these animals showed that the plane of transection, by which the animal was converted into the mid-brain condition, was rostral to the red nucleus and that the decussation of Forel was completely severed. As to the effect of the last mentioned operation we are able to confirm the observation of Rademaker that cutting the decussation of Forel results in a marked increase in the extensor rigidity but do not agree that it is the only cause in the production of the rigidity. I consider that the increase in the extensor rigidity following the section of the

decussation of Forel is due to the release of reflex arcs which influence postural tone, from the control of reflex arcs which include the nervous structures in the region of the red nucleus.

Scott (1927) showed that removal of all the cerebral cortex from cats, dogs and monkeys results in a marked extensor rigidity and suggests that decerebrate rigidity might be explained as a result of release from cortical control. The author has shown that removal of the thalamus from the thalamic cat, dog and rabbit results in an increase in the rigidity already present. The present work shows that, in rabbits, cats, dogs and guinea pigs, extirpation of the motor cortical areas for the limbs results in an extensor rigidity in the corresponding legs. Munk (1895) has also described similar observations following the ablation of the motor cortex in apes.

It appears that in the present work we are dealing with a release phenomenon; i.e., the release of the nervous mechanisms which influence postural tone from the activity of the motor cortex. Normal postural tone depends on the integrative action of the whole central nervous system. It is true that the spinal dog can stand (Sherrington, 1915) but the posture is not as stable as in the animal which has its prespinal centres intact as far cephalad as Deiters' nucleus (Bazett and Penfield, 1922). The reflex standing is still more perfect when the mid-brain and red nucleus (Rademaker, 1926) and thalamus (Laughton, 1924) are intact. It is evident that postural tone is maintained by activities of reflex arcs in the brain-stem and cord, the more important of which include Deiters' nucleus, red nucleus and thalamus. These reflex arcs are in turn influenced by activities of reflex arcs, having their centres in the cerebral cortex, in a manner which gives the finer postural adjustments. If the normal activity of any of these reflex arcs is interfered with, postural tone becomes abnormal. The present work shows that destruction of the motor cortex produces an abnormal condition in the corresponding limbs in the form of extensor rigidity.

It appears that the normal function of the motor cortex is to influence the lower reflex mechanisms, which are directly concerned in the co-ordination of movement, in such a manner that the skilled movements of the individual are produced. In the coördinated movements of animals there are postural and phasic factors. The motor cortex does not necessarily control all of the coördinated movements but plays upon the nervous mechanisms which are engaged in this function in a manner which integrates the postural and phasic factors. When the motor cortex is absent the integration between the postural and phasic factors is impaired and the result of this release of function is demonstrated by an exaggeration of the postural factor resulting in an extensor rigidity in the corresponding parts.

The symptoms of paralysis and the subsequent recovery are in accord

with the observations of Leyton and Sherrington (1917) following the removal of the motor cortex in apes. The observation that paralysis and rigidity persist for a longer period in the fore-leg than in the hind-leg of the same animal is in all probability due to the fact that the fore-legs are able to perform more highly specialized movements and hence are more directly under cortical control. In rabbits, paralysis is not so profound nor is the rigidity so intense following extirpation of the motor cortex as it is in cats and dogs. The rabbit is much lower in the phylogenetic scale than the cat and dog and cephalization has not advanced so far (Coghill, 1909). The result is that the lower reflex mechanisms in the brain-stem and spinal cord are not so dependent on cortical control and their release from cortical influences is not followed by such profound effects.

I wish to express my appreciation to Dr. F. R. Miller and Dr. P. S. McKibben for their helpful criticisms throughout this work.

SUMMARY

1. Unilateral extirpation of the motor cortical limb areas in the cat and dog results in extensor rigidity in the contralateral legs.

2. Unilateral extirpation of the fore-leg area alone in cats, dogs, rabbits and guinea pigs results in extensor rigidity in the contralateral fore-leg.

3. Unilateral extirpation of the hind-leg area, alone, in cats and dogs results in extensor rigidity in the corresponding hind-leg.

4. Unilateral extirpation of the area frontalis in cats failed to produce any extensor rigidity in the contralateral limbs.

5. Unilateral extirpation of the motor cortical areas for the legs in rabbits, cats, dogs and guinea pigs results in definite paralysis in the corresponding limbs.

6. In rabbits, cats, dogs and guinea pigs the rigidity persists for a longer period than the paralysis following the ablation of the motor cortex.

7. Following unilateral ablation of the motor cortex for the limbs in cats and dogs the rigidity is more marked and the paralysis is more profound in the affected fore-legs than it is in the affected hind-legs.

8. Following unilateral ablation of the motor cortex for the limbs in the cat and dog, the rigidity and paralytic symptoms persist for a longer period in the fore-legs than in the hind-legs.

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THE ACTION OF EPINEPHRIN UPON THE CAPILLARIES AND FIBERS OF SKELETAL MUSCLE

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The work of Krogh has emphasized the importance of the capillaries in the control of muscle circulation. The relative number of capillaries, the great variation in the proportion of open capillaries and the large mass of muscular tissue in the body make muscle capillaries of great importance in the control of the general circulation. Krogh's work on mammals was based on fixed injected material. So far as we know, living mammalian skeletal muscle has not been studied under the microscope.

It is possible with proper illumination to observe the fibers and capillaries of living skeletal muscle under the high power of the microscope. By this method many things have been seen which would have been impossible to detect otherwise.

METHODS. Cats under the influence of ether, urethane, chloralose or else decerebrated were used for our observations. Sherrington's method was used in decerebration.

The muscle to be observed, usually the sartorius, was illuminated by carrying light through a vertical glass rod as shown in the figure. Incisions were made through the skin of the hind leg in two places, one above the muscle to be observed and the other below. The rod was passed through the latter opening, then upward between the muscles until it touched the muscle to be studied.

Since this apparatus was constructed we have found that Basler employed a glass rod drawn out to small size to transmit light to the frog's gastrocnemius muscle. However with his apparatus the stage of the microscope would prevent observation upon a large animal.

Adrenalin chloride (Parke, Davis & Co.) 1:1000 solution, was diluted with distilled water for injection. Injections were made into the jugular vein.

In many of the experiments the vessels were measured by an eye-piece micrometer. The 16 mm., 4 mm. and in a few instances the 1.8 mm. objectives were used.

The anterior tibial muscle was observed in ten experiments while the sartorius was employed in the others.

OBSERVATIONS. Seventy cats have been studied. They ranged in age from ten days to undetermined adults. By weight they varied between 114 grams and 4100 grams. Nineteen were less than 1000 grams. Young cats were more satisfactory because there was less connective tissue to interfere with microscopic examination. No distinction was found between the reactions in males and females, even pregnant females.

INFLUENCE OF ANESTHETICS. *Ether* causes marked dilatation of the capillaries and an increase in the rate of blood flow. The whole field becomes hazy and indistinct as anesthesia deepens. It is possible for the experienced observer to tell by the change in the appearance of the field whether ether is being administered or not. Ether was always discontinued a short time before adrenalin was injected and was not started again until the observations for that injection were completed. Ether vapor applied locally to the field under observation caused dilatation of the capillaries and a clouding of the field. Therefore its clouding effect might be explained by peripheral action.

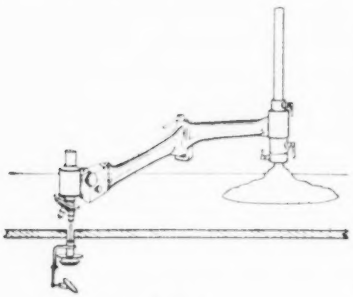

Fig. 1

Urethane when used as an anesthetic (2 grams per kilo body weight) did not seem to affect the reactions materially except that the muscular vibrations due to adrenalin were less pronounced and less constant in occurrence.

The reactions to adrenalin under *chloralose* were similar to those under ether. The reflex muscular contraction which sometimes occurred made this anesthetic unsuitable.

Vascular reactions. Small doses of adrenalin (0.2 to 0.4 cc., 1:100,000) caused first an increase in the rate of blood flow, then many new capillaries appeared while others already open, dilated. This continued up to four minutes or more after the injection in some instances. Later there might be a congestion and a slower rate of flow. The venules often reacted very much like capillaries. Accompanying the vascular changes, the whole field became clearer and brighter. Cross striations in the muscle fibers became visible or better defined. Also transverse twitching or vibration of the muscle fibers might appear. Usually in five to seven minutes the vessels began to return to normal.

With larger doses (1 cc. or more of 1:100,000) the very small vessels dilated as before while the veins and arteries constricted. The arterioles might be included in this constriction. In some individuals the capillaries on first inspection seemed constricted but with proper illumination these proved to be dilated because the walls could be seen. This appearance

was given because the corpuscles were kept from entering by constriction of the arteries and arterioles, the latter sometimes closing completely. One could never be sure of a constricted capillary unless the walls could be seen.

Constriction of the capillaries with doses even as large as 0.5 to 1 cc. 1:10,000 was rare. The beading of veins due to constriction spaced at intervals along their course was observed a number of times. Thus 2 cc. of 1:100,000 adrenalin caused a vein with a uniform diameter of 105.6μ to constrict with a beaded effect to alternate diameters of 88 μ and 35.2μ along the course.

The accompanying tables from experiments 52 and 62 illustrate the actual changes found in experiments in which measurements were made.

Experiment 52. Cat. 1825 grams. Ether

ADRENALIN INJECTED	BLOOD VESSEL	DIAMETER		SECONDS AFTER INJECTION
		Before injection	After injection	
		μ	μ	
0.5 cc. 1:100,000	Artery	26.4	29.9	125
	Vein	52.8	70.4	145
	Venule A	44.0	75.68	160
	Venule B	26.4	26.4	170
	Venule C	35.2	70.4	210
1.0 cc. 1:100,000	Vein	96.8	61.6	120
	Venule A	26.4	35.2	340
	Venule B	52.8	44.0	210
	Arteriole	26.4	17.6	108
	Capillary	4.1	6.1	70
0.5 cc. 1:10,000	Vein	61.6	61.6	215
	Arteriole	26.4	17.6	60
	Venule A	44.0	44.0	90
	Venule B	26.4	38.7	100

Reaction of muscle fibers. Brief mention has already been made of the changes which occur in muscle fibers after adrenalin injections. These are so striking that they warrant further consideration. The increase in transparency together with the better definition of the structures impresses one as much as any other change which occurs. This reaction appears to be general for it has been observed in the villi of the small intestine and in the wall of the urinary bladder. The phenomenon is most pronounced in kittens although it is present normally in cats of all ages.

The amount of adrenalin injected determines the magnitude and duration of the effect. The larger the dose up to a certain point, the greater is the effect.

Experiment 62. Cat. 1700 grams. Ether

ADRENALIN INJECTED	BLOOD VESSEL	DIAMETER		SECONDS AFTER INJECTION
		Before injection	After injection	
		μ	μ	
0.2 cc. 1:100,000	Vein	70.4	79.2	205
	Artery	44.0	47.5	205
	Venule	26.4	35.2	255
1.0 cc. 1:100,000	Vein	88.0	79.2	100
	Artery	44.0	35.2	100
	Venule	35.2	26.4	100
	Capillary A	4.1	6.97	60
	Capillary B	4.1	8.2	60
2.0 cc. 1:100,000	Vein	88.0	52.8	70
	Artery	52.8	29.9	55
	Venule	35.2	26.4	85

It has been noted that ether makes the field hazier and more opaque. Adrenalin injections oppose these effects and make the animal recover earlier from the influence of ether. This was noticed particularly in kittens although it was present in cats of all ages.

The practice has been to give ether until the animal was completely relaxed, then discontinue when the injection and observations were made. The animal soon (much earlier in kittens) recovered from the ether after injecting even a small dose of adrenalin. The larger the dose, the earlier the recovery. Furthermore the animal did not become anesthetized as easily after adrenalin had been given. Sometimes after large doses it was almost impossible to anesthetize for several minutes.

The "transparency" reaction does not depend upon the ether or any other anesthetic for its appearance. It occurs in decerebrate animals free from ether. Occasionally another phenomenon occurred in response to adrenalin injections. The muscle fibers became alternately light and dark. That is, some fibers were opaque while others were bright. In a short time these fibers changed, the dark ones becoming light and the light ones dark.

The muscular twitching resulting from adrenalin injections is always characteristic. It occurs at right angles to the longitudinal axis of the fiber and is very rapid and of short excursion. The field always becomes clearer and the fibers more distinct during and after such twitching. The twitching is sometimes preceded by vibration of the fibers which appears to be similar to the twitching except that the excursions are shorter and the movement more rapid. The inexperienced observer usually does not notice the movement of the fibers when it is of the vibration character

because of the rapidity. It merely appears to him as though in poor focus. Adrenalin twitching is so minute that it is visible only under the microscope usually the high power.

With small doses the twitching may be absent although the increased transparency occurs. As the dose is increased twitching begins and increases to a certain extent with increase in dose.

We have observed twitching caused by other substances but that with adrenalin is peculiar and occurs abruptly at intervals, brief pauses of little or no activity separate sudden onsets of a series of rapid twitches. These onsets are rather regularly spaced.

Seven *decerebrate* cats were used in this study, the observations being made long after the ether effects had disappeared. The vascular responses did not differ appreciably from those occurring under ether, but the vibration of the muscle fibers after adrenalin injections was sometimes so great that it interfered with the observation on the blood vessels. Thus in one cat with 1.4 cc. of 1:100,000 adrenalin, the twitching became so marked that nothing could be observed accurately 216 seconds after the injection.

Denervated muscle. If the nerve to a muscle has been cut only a few hours before, the capillaries and other vessels are so greatly dilated that adrenalin produces little effect except an increase in blood flow. In a muscle denervated only two or three hours before, ether produces extreme haziness which is never entirely cleared up by adrenalin. The field may become bright but there is still some haziness after adrenalin. The haziness may become so pronounced as to make detailed observations extremely difficult or impossible.

The twitching from adrenalin is not so pronounced in recently denervated muscle as in normal muscle. The sartorius muscle on one side was denervated by cutting the femoral nerve in the abdominal cavity five days before in one cat and ten days before in another cat.

In the "five day" cat (1.95 kgm.) two superficial veins showing regions of constriction and dilatation were seen in the denervated muscle. Under high power fibrillation of the muscle fibers was visible. There also appeared to be a larger number of open capillaries than in a normal muscle.

All doses of adrenalin injected from 0.4 cc. of 1:100,000 to 0.8 cc. of 1:10,000 caused the same effects, the greater doses merely increasing the effects. First there was an increase in the rate of flow followed by dilatation of the capillaries and arterioles, the capillaries dilating more than the arterioles. The field became brighter and the striations clearer. The flow stopped in the vessels and finally reversed in direction in some capillaries and arterioles. This occurred repeatedly. At this stage the dilatation was extreme. There was no constriction anywhere in capillaries, venules and arterioles with any dose. The arteries and veins, however, constricted. With 0.4 cc. 1:10,000 a vein measured with the eye-piece

micrometer decreased in diameter from 35.2μ to 26.4μ. With 0.8 cc. 1:10,000 an artery decreased from 88μ to 52.8μ.

The arterioles, venules and capillaries appeared to recover their normal condition suddenly.

The twitching from adrenalin injections was not observed in this animal. The denervated sartorius of the "10 day" cat (1.92 kgm.) reacted in exactly the same manner as in the previous cat. This animal was tested through the range of doses used in the other. Fibrillation of the denervated muscle was also present in this cat. With the injection of adrenalin fibrillation disappeared. The typical adrenalin twitching did not appear in either cat. The normal sartorius was tested for its reactions as a control. These reactions were like those already described for normal muscle. Ether was the anesthetic in both experiments.

Five other cats had the femoral nerve cut six to eight weeks before the observations were made. In two of these the cut ends of the nerve had united.

Muscle whose nerve has been cut for several weeks shows characteristic changes. There is an increase in the number of nuclei, a loss of definition of the striations and haziness of the boundary of the muscle fiber. Microscopic twitching of a more or less continuous and uniform character is present. At times this twitching disappears to recur again later. It is not the same as the fibrillation which can be seen with the naked eye. In these denervations of long-standing no very definite distinctions in the reactions to adrenalin could be made. Adrenalin did not clear up the haziness so well as in normal muscle although it made the field more translucent. Striations were not usually brought out. The vascular reactions were similar to those in the normal muscle except that there appeared to be a vasomotor instability. The reactions would occur suddenly and end suddenly.

Two rabbits (ether anesthesia) were studied for comparison. There appeared to be no great difference in the vascular reaction as compared with cats except that the arterioles, venules and slightly larger vessels may have constricted more. The capillaries dilated. The field became brighter and adrenalin vibration of the muscle fibers was present.

Discussion. It has long been known that injections of small amounts of epinephrin cause a fall in blood pressure. Of greater importance than this transient depressor effect is the differential reaction in the muscles and splanchnic area.

MacDonald and Schlapp have shown that if the anesthetic is absent, these small amounts of epinephrin cause only a rise in blood pressure. They used decerebrate animals. If ether were given the pressor response changed to a depressor one.

It must be borne in mind, however, that a general blood pressure reac-

tion is an algebraic summation. It was shown years ago (*Hartman; Hoskins, Gunning and Berry*) that muscle vessels dilate while splanchnic vessels constrict with small amounts of epinephrin. This was demonstrated by observations on the volume and the venous flow of the parts involved. If small amounts of epinephrin were injected the hind limb of a cat dilated. If the amounts of epinephrin were sufficiently large (a few tenths of a cubic centimeter, 1:10,000) the same limb constricted. Our observations show that capillaries and the smaller vessels are responsible for this dilatation and that the reversal in the reaction with larger doses is due to constriction of vessels larger than capillaries. These reactions occur regardless of the anesthetic or whether an anesthetic is present or not. In our experiments with decerebrate cats epinephrin still produced dilatation of the capillaries hours after ether had been discontinued.

When the quantity of epinephrin is sufficiently large to produce constriction in the muscle the capillaries dilate, indeed they may dilate even more but the larger vessels, arteries, veins and arterioles constrict. The constricted volume of these vessels must be greater than the dilated volume of the capillaries. The constriction of the tributary vessels sometimes becomes so great that the corpuscles are held back, only plasma passing through the capillaries. When we first saw this phenomenon we thought that the capillaries had constricted because they had apparently disappeared. If one keeps the capillary wall in view, however, he sees that the capillaries are widely open. In other words, epinephrin in doses up to a few tenths of a cc., 1:10,000 per kilo body weight is a dilator of muscle capillaries. This covers a far greater range than anything ever attained physiologically. Indeed we have been unable to produce an undoubted capillary constriction in muscle by epinephrin injections. Epinephrin, therefore, is a dilator of the permeable part of the muscle circulation but may be either dilator or a constrictor of other parts of the circulation depending upon the amount.

We do not know whether there is an increase in the permeability of the vessel wall or not. In any case the increase in capillary blood volume together with the increased surface for exchange would speed the interchange between blood and tissues.

The translucency produced by epinephrin is suggestive of speculation. Although it frequently accompanies or precedes the twitching it does not depend upon visible contraction for its production because it may be unassociated with twitching in skeletal muscle and it occurs in the small intestine and the urinary bladder without visible contraction. It may be a change in optical density.

The peculiar vibration and twitching produced by epinephrin have thus far been observed with no other substance. It seems to be identical with that produced by sympathetic nerve stimulation. These movements are

very rapid and microscopic in character. They may possibly accompany the increased heat production from epinephrin (Boothby and Sandiford).

We wish to thank J. Abramoff, J. A. Comella, L. M. Lockie, W. S. Smith and G. W. Thorne for assistance in this research.

SUMMARY

Seventy cats of various ages and two rabbits were studied while under the influence of ether, urethane or else decerebrated.

The microscopic changes of either the sartorius or the tibialis anticus were observed with transmitted light. Adrenalin ranging from 0.2 cc. 1:100,000 to 1 cc. 1:10,000 was injected into the jugular vein.

Ether caused a marked dilatation of the capillaries, an increase in blood flow and haziness of the field. Adrenalin caused dilatation of capillaries, venules, arterioles, veins and arteries and an increase in blood flow with small doses. Later congestion and a slower rate of flow might occur. The whole field became clearer and brighter. Cross striations in the muscle fibers became visible or better defined. Also transverse twitching or vibration of the muscle fibers might appear. These effects lasted as long as seven minutes.

With larger doses (1 cc. or more of 1:100,000) the capillaries and very small vessels dilated as before while the veins, arteries and arterioles constricted. The transparency and twitching became more marked.

Muscle denervated only a few hours shows little change in the capillaries in response to adrenalin because dilatation is already great.

If several days elapsed after denervation the adrenalin reaction resembled that in the normal except that it was unstable. Adrenalin twitching was absent or poorly developed.

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EFFECT OF SYMPATHETIC NERVE STIMULATION UPON THE CAPILLARIES AND FIBERS OF SKELETAL MUSCLE

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The function of the sympathetic nervous system in relation to skeletal muscle is not understood. Boeke and de Boer observed a slight decrease in muscle tone after cutting the sympathetic nerve. An increased tone from sympathetic stimulation has been difficult to demonstrate. Sherrington cut the ventral roots of the nerves to the posterior limb (cat) and the dorsal roots proximal to the ganglia. After degeneration, he obtained a slight contraction in the hind limb by stimulation of the sciatic nerve. Sherrington thought that this was due to the contraction of the "intrafusal fibers of the neuromuscular spindles."

Langworthy cut both hypoglossal nerves. After degeneration he stimulated the lingual nerve. There was a great increase in the fibrillary contraction together with great vasodilatation of the tongue. He observed in addition a slow wavy contraction of the musculature. It was suggested that the contraction was due to stimulation of accessory motor endings whose fibers are in the lingual nerve. If true it demonstrated autonomic innervation of skeletal muscle. The results which we (Hartman, Evans and Walker) obtained with epinephrin suggested experiments with sympathetic stimulation.

A microscope with a special illuminating device described in the previous communication was used for this work.

Cats were employed exclusively. Observations were upon the sartorius. The sympathetic chain in the abdomen was stimulated by means of small shielded electrodes. A large induction coil was used for the indirect current. Two small dry cells furnished the primary current. We varied the rate of stimulation as well as the strength in some experiments.

OBSERVATIONS. Fifteen cats have been studied. Nine were anesthetized with ether, two with urethane and four were decerebrated.

In the first animal very positive results were obtained by stimulating the abdominal sympathetic chain with small shielded electrodes. Stimulation for several seconds with the coil at 300 mm. caused an increase in the blood flow and an increase in the opacity of the field. At 250 mm.

and 10 to 25 seconds' stimulation the results were similar, but in addition new capillaries were seen to open and open ones dilated. At 225 mm. and 10 seconds' stimulation there was a tremendous increase in blood flow and the dilatation became so marked in both capillaries and venules that the flow stopped. The effects persisted one minute after stimulation ceased.

At 200 mm. for 10 seconds there was a distinct difference in the reaction, although the vascular changes were the same. The muscle fibers began to twitch. The striations became distinct and the field was much clearer. These observations were all under the 4 mm. objective.

Similar results were obtained in other individuals except that in some the increase in opacity or haziness did not develop or might develop during the early part of the stimulation to be followed by a much clearer field than the original.

PROTOCOL B E

Stimulation of sympathetic trunk

Cat 2.1 kgm. Right adrenal removed 10 days before. Left adrenal removed 45 minutes before.

DISTANCE OF SECONDARY COIL FROM PRIMARY	DURATION OF STIMULATION	4 MM. OBJECTIVE. RESPONSE IN SARTORIUS
mm.	*seconds*	
300		Increased velocity in capillaries
200	45	Striations clearer, field brighter
100	20	In about 60 seconds, twitching which lasted 30 seconds, after which striations and field much clearer
90	15	In 15 seconds fine, rapid almost invisible vibrations which waxed greater, becoming twitches, and then passed as they came. Twitching periodic
80	20	In 22 seconds corpuscles disappeared from capillaries, but capillaries dilated 30 seconds, vibration 55 seconds, vibration replaced by twitching 130 seconds, field much clearer 240 seconds, twitching stopped
50	15	In 15 seconds vibrations begin; vessels seem to vibrate on account of muscle fibers 55 seconds, twitching strongly. At first, periods of twitching very frequent and short; as time passed intervals longer and intensity and duration of twitching much decreased
0	10	In 10 seconds field very clear, many capillaries and venules opened. Twitching became so strong in 95 seconds that it tired the eyes to follow

The reactions in decerebrate cats were like those under the influence of ether. Frequently the sympathetic chain was cut just below the diaphragm and its central connections cut for a few centimeters so that it could be kept free from the accumulating fluid in the abdomen while being stimulated. This did not appreciably affect the results.

In order to rule out a possible increase in the output of epinephrin from the adrenals, both of these glands were removed in five experiments. The reaction to sympathetic stimulation was the same as with the adrenals present. The condensed protocol (B E) as shown on preceding page is typical.

In experiment B E little attention was paid to the vascular reaction because it is difficult to carefully follow both vessels and fibers at the same time. The vascular reaction, however, was typical.

In one cat twenty seconds' stimulation with the coil at 400 mm. caused an increase in the diameter of a capillary from 3.28μ to 4.92μ. The coil at 300 mm. caused an increase in capillary diameter from 4.1μ to 8.2μ. In another cat the field became brighter and more opaque in cycles.

In two animals the intact femoral nerve was stimulated before it entered the sartorius. The following results are typical (no. 44).

Stimulation of femoral nerve

No. 44, cat, ♀, 1.14 kgm. Ether. Both adrenals removed

DISTANCE OF SECONDARY COIL FROM PRIMARY	DURATION OF STIMULATION	4 MM. OBJECTIVE. RESPONSE IN SARTORIUS
mm.	*seconds*	
400	30	15 seconds, striations of fibers very clear 20 seconds, increase in blood flow 25 seconds, capillaries dilate 35 seconds, capillary dilatation more pronounced 55 seconds, new capillaries open
Femoral nerve cut		10 seconds later, field became extremely bright 20 seconds marked increase in circulation and dilatation of all vessels 30 seconds, peculiar continuous twitching
30 minutes later		
400	60	Same as before cutting nerve
200	120	10 seconds, violent tranverse twitching 30 seconds, circulation slower, field brighter 85 seconds, circulation almost stopped 130 seconds, dilatation of capillaries and increase in blood flow

The results of sympathetic stimulation were similar to the epinephrin responses. Circulation rate increases, capillaries dilate, and the field

becomes clearer. The muscle fibers vibrate and twitch in a similar fashion to that produced by epinephrin.

DISCUSSION. The conception of tone in muscle is perhaps best described as a constant state of contraction in which the muscle maintains a slightly shortened condition. Such a contraction is very small and it seems to improve the muscle for more vigorous contraction.

We have been able to demonstrate in this work that the sympathetic can produce rapid microscopic contractions in skeletal muscle. Although these contractions seem merely to cause transverse movement of the muscle fiber they should tend to shorten the fiber. The excursion is so small that such a shortening would be difficult to measure by any ordinary means. If this fiber movement is the sympathetic muscular "tone" it is not surprising that negative results have been obtained by some in attempting to prove its existence.

The improved response from a muscle with increased tone might be accounted for by both increased microscopic activity and improved circulation.

If this interpretation is correct, both epinephrin and sympathetic stimulation can increase muscular tone. It should be emphasized that we have been unable to produce contraction of muscle capillaries by sympathetic stimulation.

SUMMARY

Fifteen cats under the influence of ether, urethane or after decerebration, were studied.

The fibers and blood vessels were observed under the microscope by the use of transmitted light. The abdominal sympathetic chain has been stimulated by rapidly repeated induction shocks with both the adrenals intact and with the adrenals removed.

The typical effect in either instance follows:

Capillaries dilate and the rate of flow increases. The field usually becomes clearer and the striations more distinct although with weak stimulation it may become cloudier. A peculiar transverse vibration of the fibers develops to pass later into transverse twitching.

Both the vascular reaction and the muscle fiber reactions resemble the responses to epinephrin.

The fiber vibration or twitching may be muscular "tone."

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A STUDY OF THE INORGANIC CONSTITUENTS OF THE BODY WITH RESPECT TO THEIR STIMULATING EFFECT ON SUGAR METABOLISM

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The respiratory quotient is the index usually used to the amount of sugar metabolized in the higher animals and man, a rise in the quotient indicating an increase and a fall a decrease in sugar utilization. In this investigation, sugar metabolism was determined directly as well as the effect of certain substances on the rate of utilization. This was done by introducing gold fish and the substances the effect of which was being studied into sugar solutions and running sugar determinations from time to time of portions of the solutions. The effect of the chlorides, phosphates, carbonates and sulphates of sodium, potassium, calcium and magnesium on the rate of utilization of dextrose was studied. Benedict's method was employed for making the sugar determinations.

One tenth per cent solution of dextrose was prepared and 100 cc. portions were introduced into beakers. Two hundred milligrams of each of the following salts, sodium chloride, sodium bi-carbonate, di-sodium phosphate, magnesium sulphate, magnesium chloride, calcium chloride, calcium carbonate, di-calcium phosphate and sodium sulphate were weighed out and introduced into the sugar solutions in the beakers. One hundred fifty milligrams of each of the following, di-potassium phosphate, potassium bi-carbonate, potassium sulphate and potassium chloride were also weighed out and dissolved in the sugar solutions in other beakers. Two gold fish with a combined weight of approximately 5 grams were introduced into each of the beakers. One beaker of sugar solution to which no salt was added served for control. Smaller amounts of the potassium salts were used because of their greater toxicity. It was found that 200 mgm. of the potassium salts killed some of the fish during the experiments. Air was kept bubbling through the solutions to insure an adequate supply of oxygen to the fish. Sugar determinations were made immediately and at the end of thirty hours of the solutions in the beakers in which the fish were kept. At the end of the experiment water was added to replace that lost by evaporation during the experiment. The results of the average of five series of experiments are given in figure 1.

It will be seen that the control used 33 per cent of the sugar in thirty hours, and that none of the inorganic salts produced very much increase in sugar utilization except the di-sodium and the di-potassium phosphates. The di-potassium phosphate produced a very large increase in sugar utilization and the di-sodium phosphate only a slight increase. The decrease in sugar utilization brought about by magnesium sulphate is attributed to the anesthetic action of this salt. We have performed a number of experiments similar to the preceding with varying amounts of the different salts and obtained essentially the same results. As much as 700 mgm. of sodium chloride were used without any effect on sugar utilization. This confirms

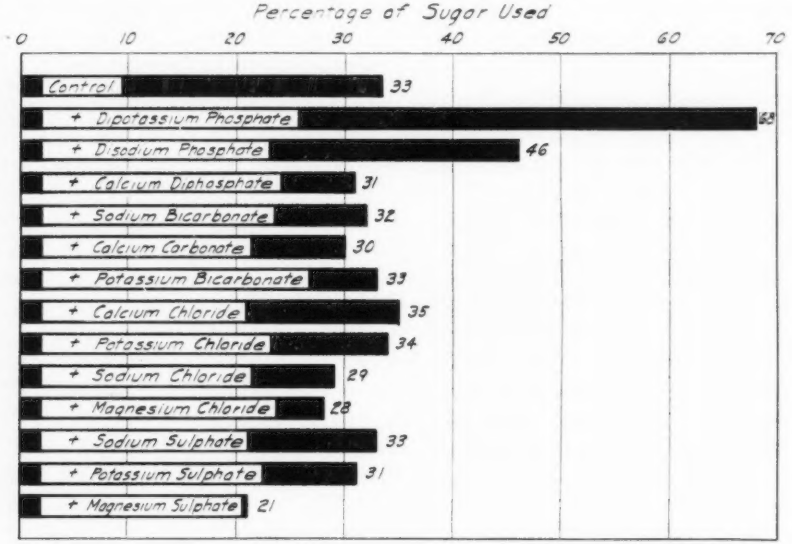

Fig. 1. Chart showing that of the inorganic constituents di-potassium phosphate is the one that produces the greatest increase in sugar utilization.

an observation of Lusk (1) that sodium chloride has no effect on the respiratory metabolism. Since the calcium carbonate and di-calcium phosphate are insoluble they were not expected to have any effect as was found to be the case.

It is known that the potassium salts are found principally in the solid constituents or cells of the body, in the form of phosphates while the sodium salts are found in the liquids of the body in the form of chlorides. It is also known that oxidation or utilization of sugar takes place in the cells particularly the muscles where the potassium phosphate occurs. The conclusion that suggests itself is that probably the potassium phosphate functions in accelerating the utilization of sugar in the muscles.

The question that might be raised in this connection is, will the results obtained on sugar utilization with gold fish in this investigation hold for the higher animals and man. It is probable that they will for insulin has the same effect on sugar utilization in gold fish that it has in man. We found that the addition of a small amount of insulin to the sugar solutions containing the fish increased sugar utilization. It was also found that if the gold fish were placed in 100 cc. of water in which there was a large amount of insulin, 100 units or more, they soon went into convulsions just as is the case with the administration of overdoses of insulin to higher animals and man. If the fish were removed while in this state, to a 0.2 per cent dextrose solution, the convulsive movements ceased in a short time and the fish swam around again in a normal fashion.

The effect of the inorganic salts on the metabolism of sugar by paramecium caudatum was also studied. The paramecia were collected and washed free of debris by means of a small centrifugalizing machine. The centrifugalizing tubes were graduated in cubic centimeters so that the paramecia were measured as they were collected. Five cubic centimeters of paramecia were introduced into sugar solutions in the beakers to which the salts had been added, just as was done with the fish. The di-potassium phosphate greatly increased sugar utilization by paramecia similarly to what was found to be the case with the fish. Insulin also increased the rate of sugar utilization by paramecium.

SUMMARY

1. The effect of the various inorganic salts occurring in the body on sugar utilization was determined directly.

2. It was found that of these inorganic salts di-potassium phosphate produced a very marked increase in sugar utilization, di-sodium phosphate a small increase while the other inorganic constituents except magnesium sulphate had no effect. The magnesium sulphate decreased sugar utilization due probably to the anesthetic effect of this salt.

3. Since the phosphate of potassium occurs in the solids and hence in the muscles, the place where sugar metabolism is greatest, it is assumed that this salt functions in some way in accelerating the utilization of sugar there.

4. It was also found that small amounts of insulin increased sugar utilization and that excessive amounts produced convulsions which ceased upon removal of the fish to sugar solutions.

5. Of the inorganic salts, the di-potassium phosphate was the one found to increase greatly sugar utilization in paramecium caudatum just as it did in the gold fish. Insulin also increased sugar utilization in paramecium.

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EFFECT OF DIFFERENT AMOUNTS OF SEXUAL INDULGENCE IN THE ALBINO RAT

VI. Life Span and Cause of Death[1]

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In the first of this series of papers on the effect of various amounts of sexual indulgence (Slonaker, 1927a) we described in detail the plan and method of procedure of this experiment. In this paper, the last of the series, a brief summary of the preceding papers will be given.

The 126 albino rats were segregated into five groups and each group was allowed a certain amount of sexual indulgence. Group A was composed of 29 pure virgins and 6 strict bachelors which were never permitted any sexual intercourse nor any intermingling with the opposite sex. Group B consisted of light breeders (9 females and 4 males) whose sexual indulgence resulted in not less than two nor more than three litters during their life span. Group C (9 females and 4 males) was designated medium breeders and represented normal conditions as nearly as possible. This group produced and reared as many litters as possible during their life. The females of this group spent the greater part of their sexual lives in gestation and nursing their young. Group D (9 females and 4 males) was composed of heavy breeders whose young were destroyed at birth and the pairs remated as soon and as often as possible as long as they lived. Group E consisted of 40 hysterectomized females and 12 normal males which were kept constantly together. They were segregated into four groups, three males with ten females, in separate large cages. Since the removal of the uterus does not interfere with the normal oestrual cycles in the rat these animals had excessive sexual indulgence without the possibility of bearing young. Twelve hysterectomized females having no sexual indulgence were used as controls for this group. Five females from each of the groups A, B, C and D were confined throughout their life span in individual revolving cages to ascertain their spontaneous activity, daily food consumption, normal oestrual cycles, gestation periods and pseudopregnancies.

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All other animals were kept in uniform stationary cages. The experiment was continued until the animals died a natural death. All animals were autopsied at death and the condition of the organs and cause of death determined as nearly as possible from macroscopic observations.

All the animals used were from the same stock of purebred albino rats and as nearly as possible the same age at the beginning of the experiment. The average age was 74.5 days and the range was from 58 to 88 days. The females averaged 73.6 days and the males 79 days. The experiment was started when but few of the animals were older than the average age of sexual maturity. The age at delivery of the first litter and the spontaneous activity records indicated that most of them were still sexually immature when the experiment began.

With the exception of the two kinds of cages used and the amounts of sexual indulgence permitted all the animals were subjected to as nearly as possible the same environment. They were all kept in the same well lighted and ventilated room which assured the same temperature and moisture conditions and were always fed at the same time daily more than they would consume between feeding times on the same well balanced synthetic food. We have therefore assumed that any differences in the results of the different groups were due to the one variable factor—the amount of sexual indulgence permitted.

I. Growth. In the first paper (Slonaker, 1927a) we discussed the effect of the different amounts of sexual indulgence on growth in the different groups. We found that the order of growth of the females, as indicated by the average maximum body weight, from the lowest to the highest was 1, group A, virgins, 254 grams; 2, group E, hysterectomized rats having excessive coitus but no breeding, 266 grams; 3, group B, light breeders (two or three litters), 298 grams; 4, group D, heavy breeders (young destroyed at birth and mothers promptly remated), 304 grams; and 5, group C, medium breeders (mothers reared as many litters as possible), 306 grams. The order of growth of the males from the least to the greatest average maximum weight was 1, group E, 333 grams; 2, group B, 338 grams; 3, group C, 340 grams; 4, group D, 362 grams; and 5, group A, 363 grams. The females showed greater differences in weight than the males. All groups of females showed a tendency to increase body weight after the menopause. There was a direct correlation between sexual indulgence and a decrease in the amount of the follicular hormone with an increase in weight of the female. Rats which had sexual indulgence showed a tendency to grow more rapidly in early life than abstainers. The bearing of young at an early age appeared to inhibit body growth in later life.

II. Activity. In regard to the effect on spontaneous activity (Slonaker, 1927b) we found that during the whole life span the total average distance run by the females of the different groups in the order of accomplishment

was 1, group A, virgins, 5989 miles; 2, group B, light breeders, 3851 miles; 3, group D, heavy breeders, 2468 miles; and 4, group C, medium breeders, 1696 miles. The total average run was in reverse order to the number of litters born and nursed. If a sufficient time were allowed to intervene between litters, as in group B, an average daily activity equal to that of the normal virgin, of the same age, was attained. The mere bearing of young (group D) did not reduce spontaneous activity as much as delivering the same number of litters and nursing them (group C). The activity after the menopause was not affected by the different amounts of sexual indulgence tried. There was a close correlation between activity and growth. The most active group reached the lowest maximum weight and the least active the greatest maximum weight.

III. Food consumption. When the food consumption was considered we found a noticeable difference in the groups which we were able to test (Slonaker, 1928a). The average total food intake for the different groups from the least to the greatest was 1, group A, virgins, 14,749 grams, or 58,107 calories; 2, group C, medium breeders, 15,766 grams, or 62,906 calories; 3, group D, heavy breeders, 16,209 grams, or 63,864 calories; and 4, group B, light breeders, 17,176 grams, or 67,571 calories. This was an average daily intake of 15.64, 17.05, 17.73 and 17.68 grams, or 61.62, 67.18, 69.86 and 69.66 calories respectively. The average intake of food for activity in virgin rats was 0.005536 gram, or 0.02181 calorie for each gram of body weight per mile run. During the first twenty days of gestation the average daily food consumption was increased 2 grams (7.98 calories) and during the first twenty days of lactation the intake averaged 9 grams (35.46 calories) more than in non-pregnant rats of the same age. Prenatal growth of the young thus required a less expenditure of energy on the part of the mother than postnatal growth. The amount of food required by the mother for the development of each young from the beginning of gestation to the weaning age was approximately 97.35 grams, or 383.56 calories. For gestation alone it was 20.7 grams, or 81.56 calories. The increased food consumption during gestation and lactation was greatly augmented by the pronounced reduction in spontaneous activity. Young breeding females showed a more rapid increase in daily food consumption than young virgin rats. There was a direct correlation between the total food intake and activity, growth, and reproduction. A greater expenditure of energy for reproduction was manifested in light breeders than in medium or in heavy breeders.

IV. Length of sexual life. When the effect of the different amounts of sexual indulgence on the length of sexual life was studied some very pronounced differences were noted (Slonaker, 1928b). It was found that in all groups the first productive oestrus demonstrated by mating and delivery of young occurred at a later age than the first oestrus indicated by

vaginal smears and activity tests. It was also found that the last productive oestrus which resulted in mating and birth of young occurred at an earlier age than the last oestrus indicated by vaginal smears and activity curves. The rhythmic cycles of activity, which are associated with oestrus, ceased at an earlier age in the virgins than in any of the breeding groups. The average length of seuxal life as indicated by productive matings of the groups whose sexes were together was group B, 544 days; group C, 525 days; group D, 342 days. The discarding of the young at birth followed by prompt remating (group D) caused a shifting of reproductive activities toward early life and an early sterility of the female. The number of litters born appeared to be as great a factor, if not a greater one, than age in determining the length of reproductive activity. That is, after a certain number of litters had been delivered the female failed in later successful matings to deliver young even though opportunity was constantly present and the animals were 200 or more days younger than the average age at the menopause and about 145 days younger than the average age in group C at last productive mating.

The number of successful copulations, as indicated by sperm in the vaginal smear, which resulted in pregnancy and the delivery of young was 57 per cent in group B, 50 per cent in group C, and 34 per cent in group D. The other matings resulted in pseudopregnancy. Fertility tests in later sexual life showed that in group B 75 per cent of the males were fertile at the age of 581 days and 75 per cent of the females were fertile at 635 days; in group C 50 per cent of the males at 609 days and 100 per cent of the females at 625 days were fertile; in group D 50 per cent of the males were fertile at the age of 591 days and 100 per cent of the females had their last productive mating at the average age of 456 days.

V. Offspring. In regard to the effect on the offspring (Slonaker, 1928c) it was found that the size of the successive litters of group B was normal. That is, the second litter was larger than the first and the last litter was the smallest. But in groups C and D, which delivered as many litters as possible, the first litter averaged the largest and the successive litters became gradually smaller. Mothers of a given age which nursed their young had larger litters than mothers of like age whose young had been continually destroyed at birth. The cessation of reproductive activities in group B for 223 days was followed by the delivery of smaller litters later in life than those thrown by group C at the same age. The average weight of the young at birth was heaviest in group B and lightest in group D. At weaning the young of group B were heavier than those of group C. Both birth and weaning weights were in indirect correlation with litter size. The per cent of mortality of the young was slightly greater in group B than in group C. In both groups the mortality of the males was less than that of the females during the nursing period. In

stillbirths and those which died at birth there were more males than females. These results indicate that the vitality of the male was less than that of the female during prenatal life, but after birth the reverse obtained. Cannibalism was only one per cent greater in group B than in group C. The average lengths of the gestation periods was found to be in group B 21 days 20.8 hours; in group C 22 days 2.2 hours; in group D 22 days 6 hours. Former experiments (Slonaker, 1925) showed a direct correlation between a reduction in litter size, advancing age and a lengthening of the gestation period. The results of this experiment are in direct opposition. The lengthening of the gestation period in group C and a still greater lengthening of it in group D has been attributed to the reduced vitality of the mother, caused by forced breeding, which resulted in slower development of the intrauterine young. The sex ratio was not noticeably affected and was below normal in all groups.

VI. Total life span and cause of death. When the total life span is considered we find some noticeable differences. In figure 1 we have shown the average length of life (heavy line) and the minima and maxima for each sex in the different groups.

The females of group A (virgins) had an average life span of 890 days. The shortest life was 466 days and the longest was 1357 days, a range of 891 days. This old age greatly surpassed the oldest age attained by either sex of any of the other groups and almost equaled the oldest age of 1362 days formerly recorded for a male rat (Slonaker, 1912). Approximately 83 per cent of the deaths occurred between the ages of 700 and 1100 days. The distribution was 13.80 per cent between 700 and 800 days; 20.65 per cent between 800 and 900 days; 24.15 per cent between 900 and 1000 days; and 24.15 per cent between 1000 and 1100 days.

In group B, light breeders, the females reached an average age of 981 days which was the oldest average age attained by this sex in any of the groups. The earliest death, which took place at a later date than in any other group, occurred at the age of 827 days and the latest death at 1118 days. This gave a range of 291 days which was 600 days less than in group A. The percentage distribution was 22.2 per cent between 800 and 900 days; 33.35 per cent between 900 and 1000 days; 33.35 per cent between 1000 and 1100 days; and 11.1 per cent between 1100 and 1200 days. Approximately 89 per cent of the deaths occurred between the ages of 800 and 1100 days.

The females of group C, medium breeders, had an average life span of 923 days, which was the second greatest average of any groups. The minimum and maximum ages were 663 and 1115 days respectively. This gave a range of 452 days. Fifty-six per cent of the deaths occurred between the ages of 900 and 1100 days.

The average age of the females in group D, heavy breeders, was 906

days. This was but sixteen days greater than that of the virgins. It was 17 days less than the average of the females of group C and 75 days less than in group B. The range of 288 days was between 726 days and 1014 days. The percentage distribution of the deaths was 22 per cent between 700 and 800 days; 22 per cent between 800 and 900 days; 11 per cent between 900 and 1000 days; and 45 per cent between 1000 and 1100 days. The removal of young at birth not only caused a shifting of reproductive activities toward early life but also appears to have shortened the average life span as compared with the other breeding groups.

The females of group E, which was composed of hysterectomized rats confined with normal males and having excessive sexual indulgence without bearing young, had an average length of life of 817 days. This was the shortest of any of the groups. The range between the minimum, 478 days, and the maximum, 1198 days, was 720 days. This range was exceeded only by group A. There was no very pronounced grouping of deaths at any age as found in the other groups. The percentage distribution was 10 per cent between 400 and 500 days; 5 per cent between 500 and 600 days; 13 per cent between 600 and 700 days; 26 per cent between 700 and 800 days; 13 per cent between 800 and 900 days; 8 per cent between 900 and 1000 days; 10 per cent between 1000 and 1100 days; and 15 per cent between the ages of 1100 and 1200 days.

To show that the operation for hysterectomy was not the cause of this great reduction in the span of life in group E the results are given of 12 control hysterectomized rats which were operated at the same time and age as those of group E. These rats had no sexual indulgence nor intermingling with the opposite sex during their entire life. These virgins reached an average age of 922 days which was 105 days older than the average of group E. The range between the earliest death, 698 days, and the oldest age, 1357 days, was 659 days. The percentage distribution of ages at death was 8.34 per cent between 600 and 700 days; 16.66 per cent between 700 and 800 days; 33.32 per cent between 800 and 900 days; 8.34 per cent between 900 and 1000 days; and 25 per cent between the ages of 1000 and 1100 days. The extremely old female, which lived to be 1357 days of age, belonged to this group. This group of virgins showed a marked tendency toward uniformity in age at death—three-fourths of them dying between 800 and 1100 days. This shows that the operation for hysterectomy was not the cause for the shortened life span of group E and indicates that excessive coitus unaccompanied by other reproductive activities was responsible. The results also show that this group was more affected than the groups in which coitus was followed by normal reproduction.

A summary of the effect of these various amounts of sexual indulgence on the average life span of the female from the shortest span to the longest

was group E, 817 days; group A, 890 days; group D, 906 days; group C, 923 days; and group B, 981 days.

When the effect on the life span of the males is considered a very noticeable difference was observed (fig. 1). The strict bachelors, group A, had an average life span of 899 days with a range between the minimum, 639 days, and the maximum, 953 days, of 314 days. The males of this group had a shorter life span than those of any of the other groups. Group B

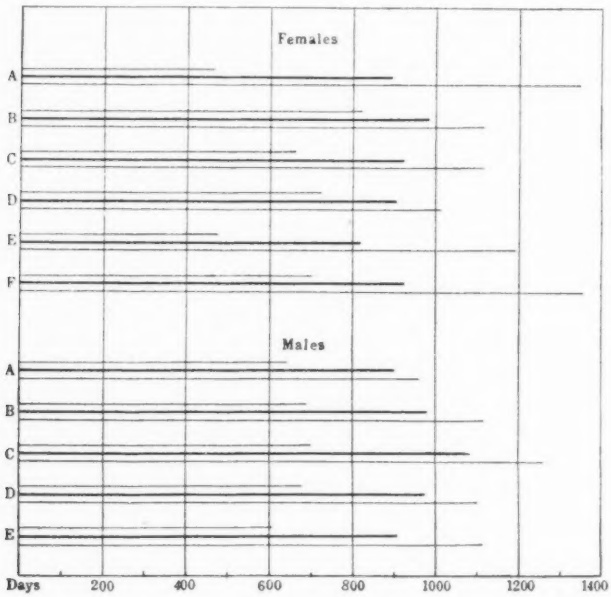

Fig. 1. Giving the average length of life in heavy lines and the minimum and maximum ages at death in light lines of the females and males of the different groups. *A*, virgins and strict bachelors; *B*, light breeders; *C*, medium breeders; *D*, heavy breeders; *E*, hysterectomized females with normal males having excessive sexual indulgence without breeding; *F*, control hysterectomized females having no sexual indulgence.

had an average span of 975 days. The range from the minimum, 684, to the maximum, 1109 days, was 425 days. Group C had an average age at death of 1079 days. The range in this group from the minimum, 698 days, to the maximum, 1259 days, was 561 days. This maximum age was the oldest found in any of the groups surpassing the next oldest, found in group E, by 149 days. The sexual activity of this group was distributed over 525 days, the average productive sexual life of the females. In group

D the average age at death was 975 days. This group had the same average span of life as group B. The range of the life cycle between the minimum, 677 days, and the maximum, 1098 days, was 421 days. In both group B and group D the sexual activities occurred relatively early in life. The results of these two groups are very similar. The average age at death was the same, and the youngest age at death and the oldest age attained were only 21 and 11 days respectively greater in group B than in group D. Both groups D and C had almost the same amount of sexual activity. In group D it took place during an average of 342 days in relatively early life, while in group C it was distributed over an average of 525 days. This seems to indicate that sexual activity early in life followed by cessation of these activities during later life resulted in a shortening of the life span of the male. The males of group E, which had excessive sexual indulgence throughout life, had an average life span of 906 days. This was but seven days longer than the shortest average life (899 days) found in the bachelors (group A). This indicates that excessive sexual indulgence distributed over the whole sexual life of the male did not greatly affect the life span when compared to normal bachelors. The range between the minimum, 604 days, and maximum age at death, 1110 days, was 506 days.

A summary of the results of the effect of various amounts of sexual indulgence on the life span of the male from the shortest average age at death to the greatest was group A, 899 days; group E, 906 days; groups B and D, 975 days; and group C, 1079 days. Group C had not only the greatest average life span but also had the oldest record of any of the groups. On the other hand the strict bachelors (group A) had not only the shortest average age at death but also the oldest age recorded for the males of this group was 145 days younger than the next oldest recorded age found in group D. These results indicate that sexual indulgence tends to prolong the life span of the male and that the amount of prolongation depends both on the extent of this activity and on the time in life during which it occurs.

Cause of death. When possible, autopsies were made at death to determine the condition of the various organs and, if possible, the cause of death. Only macroscopic examinations were made. The body weight at death was taken, the condition of the teeth and any external malformations noted, and the body and tail lengths were determined to ascertain changes in stature if any occurred. The internal organs which were especially noted were the lungs, heart, alimentary tract, pancreas, liver, spleen, kidneys, adrenals, and the reproductive organs. Many of the animals showed that more than one organ was affected, in which case each was listed as abnormal.

The organs which were found to be most commonly affected were the

lungs and the alimentary tract. In the 114 autopsies made of both sexes 43.7 per cent showed lesions of the lungs and 40 per cent had disturbances of the alimentary tract. In both cases the females showed a higher per cent than the males. The lesions of the lungs varied from a few spotted areas showing on the surface to an almost solid cheesy mass involving one or both lungs and sometimes leaving only a small portion functional. This was by far the most common lung trouble found. No bacteriological tests were made and this disease was diagnosed macroscopically as tuberculosis. About 5 per cent were classed as pneumonia. In the digestive disturbance 7 per cent was apparently due to constipation and 33 per cent was accompanied by gas accumulation. This was found principally in the small and

TABLE 1

Giving the per cent of various lesions as found at autopsy in the different groups

GROUP	LUNGS			ALIMENTARY TRACT			TUMOR					CANCER-LIKE	ABSCESS	OLD AGE
	Tuberculosis	Pneumonia	Total	Constipation	Gas	Total	Skin	Uterus	Ovary	Stomach	Total			
A. Female...	39.3	14.3	53.6	7.14	42.8	50	3.55	3.55			7.14		3.55	14.3
B. Female...	22.2		22.2	11.1	33.3	44.5	11.1				11.1			44.5
C. Female...	25		25		50	50	25	12.5		12.5	50	12.5	12.5	25
D. Female...	50		50		25	25	12.5		25		37.5	12.5	12.5	12.5
E. Female...	45.4		45.4	9.1	39.4	49.5	6.7		3.3		12.1			21.2
A. Male.....	16.6		16.6											16.6
B. Male.....	25		25	25	50	75								25
C. Male.....	50		50		25	25								50
D. Male.....	50		50		25	25								25
E. Male.....	20	20	40	10		10	10				10			30

large intestine and cecum. In some cases the stomach was also involved. Distention often occurred to several times the normal diameter making the wall very thin and transparent. This condition was apparently caused by the gas bacillus, and death was probably due to toxic poisons which were absorbed.

Tumors were developed in 13 per cent of the animals. They were more prevalent in the females than in the males. Two were uterine, three ovarian, two stomach and the others were located just under the skin and were classed as skin tumors. In a few cases these skin tumors became raw and cancer-like in appearance. This may have been due to abrasions which later became infected. All the tumors began developing rather late in the life of the animal. In some cases this abnormal growth was

relatively enormous and made it difficult for the animal to move about easily. In one animal (A3, stationary cage, virgin) whose weight at death was 309 grams an internal tumor near the stomach measured 30 × 60 × 70 mm. and weighed 95 grams. In 23 per cent of the autopsies no organic trouble could be noted and death was attributed to old age.

An attempt has been made to condense these autopsy results in table 1. This table shows that the per cent of females in the different groups which were affected with lung trouble, from the greatest to the least, was virgins 53.6 per cent; group D, 50 per cent; group E, 45.4 per cent; group C, 25 per cent; and group B, 22.2 per cent. It is significant that groups B and C, whose reproductive activities were normal, showed the lowest per cent of lung trouble and that the virgins (A), the heavy breeders (D) whose young were destroyed at birth and those having excessive coitus (E) were the most affected. In the males the order of lung trouble from the highest to the lowest was D, 50 per cent; C, 50 per cent; E, 40; B, 25 per cent; A, 16.6 per cent. This indicates that bachelors were much less prone to affections of the lungs than virgin rats and that sexual indulgence was conducive to lung trouble in the male rat.

Digestive disturbances were found to be associated with and dependent more on old age than on amounts of sexual indulgence. Tumors were found more prevalent in females than in males. The virgins had the lowest per cent of tumors and the medium and heavy breeding females had the greatest per cent. Ovarian tumors were found only in the heavy breeders (D) and in the excessive indulgence group (E). The results seem to indicate that the bearing of the greatest number of young possible was favorable to the development of tumors which usually appeared late in life or after the females had ceased to breed. Only one male developed a tumor. This was in group E. Apparently the different amounts of sexual indulgence permitted the different groups of males had little if any effect on tumor formation.

When we consider the animals in which no organic lesions were found at autopsy and which were classed as having died from senility we find in the females that the greatest per cent (44.5) was in the light breeders, group B, and the least per cent (12.5) was in the heavy breeders. The order from the lowest to the highest per cent was group D 12.5 per cent; group A, 14.3 per cent; group E 21.2 per cent; group C 25 per cent; and group B 44.5 per cent. These data again suggest what we have previously stated that the interference with normal reproduction by killing the young at birth followed by prompt remating was more injurious to the mother than allowing the young to be reared. The results also indicate that the amounts and character of the sexual indulgence permitted groups B, C and E were more conducive to a senile death than was shown by the group of strict abstainers, the virgins. In the males we find that the bachelors had

a lower per cent die from old age than in any other group. With the exception of group C which had 50 per cent die from senility there was no marked difference.

Stature. It has been stated above that the order of average maximum weights of the different groups of females from the lowest to the highest was group A 254 grams; E 266 grams; B 298 grams; D 304 grams; and C 306 grams. A similar order for the males was E 333 grams; B 338 grams; C 340 grams; D 362 grams; and A 263 grams. In order to see if these variations in body weight were associated with similar changes in stature or to accumulation of adipose tissue measurements of body length and tail length were made at autopsy. The results of these measurements show that the average body length of the females from the least to the greatest was group A, 181.2 mm.; B, 183.5 mm.; C, 184 mm.; E. 186.5 mm.; and D, 190 mm. The average for the males was group B, 180 mm.; C, 183.5 mm.; E, 190 mm.; A, 194 mm.; and D, 195 mm. There were too few males however, for these data to be reliable. The average length of the tail in the different groups of females was in the exact same order as the body weights. In the males the order was only partly in accord with body length. This was principally due to distal portions of this member having been lost in fights or accidents. These data show that the virgins had not only the smallest maximum average weight but also had the shortest body length of any of the groups of females having sexual indulgence. It is also noted that the groups which had the greatest amount of sexual indulgence had the longest body length. The males showed in general that sexual indulgence was a detriment to increased stature. The bachelors were not only the heaviest but also showed almost as great a body length as any of the groups.

SUMMARY

1. The average life span of the females of the different groups from shortest to longest was E, 817 days; A, 890 days; D, 906 days; C, 923 days; and B, 981 days. The similar order for the males was A, 899 days; E, 906 days; B and D, 975 days; and C, 1079 days.

2. Normal reproduction was more favorable to longevity of both the female and the male than chastity.

3. Excessive sexual indulgence without bearing young tended to shorten the life span of both female and male rats.

4. Destroying the young at birth and promptly remating shortened the life span of the female more than almost continuous normal reproduction.

5. Both female and male rats without sexual indulgence had shorter average life spans than the breeding groups.

6. The females were more affected than the males.

7. Diseases of the lungs of the females of the different groups from the greatest to the least was A, 53.6 per cent; D, 50 per cent; E, 45.4 per cent; C, 25 per cent; and B, 22.2 per cent.

8. The bachelors, group A, had the least lung trouble of any of the groups of males. Their order from greatest to least was C and D, 50 per cent; E, 40 per cent; B, 25 per cent and A, 16.6 per cent.

9. Tumors, which usually appeared in old age, were much more prevalent in the females than in the males. Females of groups C and D had the highest per cent and of group A had the lowest per cent.

10. Digestive disturbance was common to old rats of both sexes in all groups.

11. In the animals which had no apparent organic trouble and were classed as having died of old age group B of the females and group C of the males had the highest per cents. Both females and males of group A showed low per cents.

12. Sexual indulgence increased the average body length of the female. Their order of average body length in millimeters from least to greatest was A, 181.2; B, 183.5; C, 184; E, 186.5; D, 190. The meager data available do not indicate a like effect on the males.

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THE EFFICIENCY OF BICARBONATE AND PHOSPHATE BUFFERS FOR EXPERIMENTATION WITH EXCISED ORGANS

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The buffering of saline solutions for use with excised organs is not as simple a matter as might be supposed; for in such experiments it is necessary not only to preserve the hydrogen ion concentration, but also to avoid undesirable effects on the functional activity of the tissue by the buffer substances, either directly or indirectly, especially through alterations of the concentration of carbon dioxide and of bicarbonate and calcium ions. The most "normal" function should be secured if one could really imitate the natural buffer mechanisms of the blood; but the most important of these mechanisms are not available for artificial systems, namely, the hemoglobin and other proteins and the corpuscular exchange; nor is it feasible to procure the nice adaptation of the aeration to the hydrogen ion concentration, which the respiratory center secures in the living organism. There remain of the natural buffers only the carbonic and phosphate systems. By either or both of these it is easy to adjust the pH at any desired level of the physiologic range; but in the absence of the other natural buffers, they would need to be used in concentration so abnormal that they tend to produce alterations of function. Consequently, a saline fluid that would faithfully reproduce the ash of the blood, but without its proteins and other protective devices, could not be at all "normal" for excised tissues. A closer approach to functional normality should be obtainable by readjusting the concentration of the ions to the altered conditions.

The different degrees of aeration which are necessary for various experimental arrangements introduce further complications, especially with bicarbonate solutions. Effective buffering under such conditions is possible only by balancing the aeration with the bicarbonate concentration, hydrogen ion concentration and acid production, which would need to be worked out and adjusted with each arrangement. In some cases this is practically superfluous, for instance, for perfusion liquids; with others it is feasible but limits the range of experimentation, and this may be fatal

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to the object of the experiment; so that it is often more simple and sometimes necessary to readjust the pH periodically, instead of automatically, by the addition of carbon dioxide or more simply of bicarbonate. The aeration difficulty does not apply to bicarbonate-free phosphate buffers. For these it is only necessary that the aeration is sufficiently active to remove the carbon dioxide produced by the tissue. However, phosphate buffers introduce other complications, which are perhaps even more serious. As was pointed out by Rona and Neukirch (1912), the absence of bicarbonate is distinctly detrimental to the function of tissues; and we have found that even in the presence of bicarbonate excessive concentrations of phosphates, as in the Fleisch (1922) solutions, tend to produce alterations in the state of the calcium and therefore disturbance of the important cathion equilibrium. This is manifested by gradual precipitation, presumably preceded by a change of some of the calcium from the ionic to the colloid condition.

These principles have been pointed out before, but their practical application has not been easy; partly at least for want of quantitative data under actual working conditions. In experimenting with excised intestines we came to feel the need of such data so seriously, that it became necessary to supply them. The intestines appear especially well suited for such inquiries, because their motor activity is a rather delicate index of the functional effects of saline solutions. The conditions with other organs and arrangements are sufficiently similar, so that the principles and some of the data may be applied to them directly; or at least, they come sufficiently close so that they may shorten the scope of individual experimentation.

The present paper is concerned with the first part of the inquiry, the actual importance of the aeration factor in various experimental arrangements and for different solutions. It includes *a*, the determination of the concentration of the buffers required for the physiologic pH, at room temperature and at body temperature, with and without dextrose; *b*, the changes of the pH of the various types of solutions in the course of aeration, with the usual experimental systems; *c*, the buffer efficiency in the presence of the intestine, working under several types of conditions; and *d*, the practical arrangements for maintaining the pH by the addition of alkali or acid. The influence of buffers on functional activity will be considered later.

a. The concentration of the buffers required for the physiologic pH. "Normal," "optimal" and "maximal" functionation should not be confused. The normal function of an organ is adjusted to the efficiency of the body as a whole, and therefore need not be optimal for the organ itself. The normal, in this sense, could not be determined experimentally under the artificial conditions of excised organs; with these it means only that the

experimentor endeavors to reproduce as closely as possible the principal conditions that he conceives to exist in the living body. The maximal functionation of an excised organ, on the other hand, is amenable to experimental determination, the chief difficulty being the choice of the criterion of activity. The maximal functionation is not necessarily optimal; for it may exhaust the organ so rapidly that the total performance is diminished; or it may interfere directly with the performance of the proper function, as spasm is likely to do. The optimal functionation is therefore a relative and rather indefinite term.

The relation of the pH to the functional efficiency may conceivably vary with conditions, so that the pH that is optimal for one set of conditions may not be optimal for another, and similar variations may be presumed for the "normal" point. It should therefore not be taken for granted that the hydrogen ion of the arterial blood, the colorimetric pH of 7.3 to 7.5, which is normal for the intestinal functionation in the body, would also be the normal in the saline solutions; but it is at least a convenient point of departure for experimentation. The optimal point and range have been determined experimentally by Rona and Neukirch, with the Magnus arrangement, on a number of buffers, with consistent results. They found the best functional activity about 7.3; with increase of acidity, the activity declined, became quite small about 6.3, and arrested about 5.6. With increase of alkalinity, definite decrease of function occurred by 8.2 and arrest about 8.8. In our experience, the functionation in bicarbonate and phosphate solutions remains good between pH 7.3 and 7.9, and deteriorates beyond these limits, so that we would consider this as an optimal zone. The first task therefore was to determine which concentrations of bicarbonate and phosphate buffers come within this range. The observations were confined to the Locke-Ringer saline solution containing, per cent: 0.92 NaCl; 0.042 KCl; 0.024 crystallized $CaCl_2 \cdot 2H_2O$. It should be understood that the present data apply to this solution only, and that they may not be transferred directly to others, since variations in the calcium content affect the pH of buffer mixtures. When prepared with our ordinary stock distilled water, this solution had the pH 6.6. The acidity is due to the absorption of carbon dioxide by the distilled water, as pointed out by I. P. Williams and Sweet (1922); for when prepared with redistilled and boiled water, the pH of the solution was 7.0. However, since the water-acidity is important only in unbuffered solutions, the buffered solutions were ordinarily made with the stock distilled water, but in the following tests of buffer efficiency, the redistilled water was used. Various amounts of sodium bicarbonate, disodium phosphate and monosodium phosphate were added to the plain Locke-Ringer solution of pH 6.9 to 7.0, and the hydrogen ion concentration of the freshly prepared solutions was determined colorimetrically at 25° and

38°C., by comparison with the standardized buffers of Palitzsch (borax: boric acid) and of McIlvaine (disodium phosphate:citric acid), using phenol red and cresol red as indicators. The entire system was checked by the potentiometric method through the kindness of Dr. M. G. Banus.

Table 1 gives the composition and the hydrogen ion concentration of the solutions at 25°C. The variations in the bicarbonate solutions are due to the use of several specimens of the salt. Warming to 38° increased the alkalinity of the solutions by about 0.1 pH, which may be considered practically immaterial. The addition of 0.1 per cent of dextrose did not

TABLE 1

The pH of buffered Locke solutions

NUMBER OF SOLUTION	COMPOSITION OF THE SOLUTION, PER CENT	pH AT 25°C.
O	$NaCl = 0.92$, $KCl = 0.042$ $CaCl_2 \cdot 2H_2O = 0.024$	6.9–7.0
I	O + $NaHCO_3 = 0.004$	7.5
II	O + $NaHCO_3 = 0.008$	7.5–7.6
III	O + $NaHCO_3 = 0.015$	7.8
IV	O + $NaHCO_3 = 0.03$	7.7–7.9
V	O + $NaHCO_3 = 0.045$	8.3
VI	O + $Na_2HPO_4 \cdot 12\ H_2O = 0.008$	7.2
VII	O + $Na_2HPO_4 \cdot 12\ H_2O = 0.016$	7.5
VIII	O + $Na_2HPO_4 \cdot 12\ H_2O = 0.024$	7.6
IX	O + $Na_2HPO_4 \cdot 12\ H_2O = 0.016$ $NaHCO_3 = 0.008$	7.7
X	O + $Na_2HPO_4 \cdot 12\ H_2O = 0.024$ $NaHCO_3 = 0.015$	7.9
XI	O + $Na_2HPO_4 \cdot 12\ H_2O = 0.016$ $NaHCO_3 = 0.03$	8.1
XII	O + $NaH_2PO_4 \cdot 12\ H_2O = 0.004$ $NaHCO_3 = 0.03$	7.5–7.6
XIII	Fleisch solution containing $NaCl = 1.0$, $KCl = 0.05$, $CaCl_2 = 0.03$, $MgCl_2 = 0.01$, $H_2PO_4 = 0.049$, $Na_2CO_3 = 0.05$	7.5

alter the pH of any of the solutions. The table may therefore be considered as applying to the ordinary uses of these solutions. It shows that the desired range of pH 7.3 to 7.9 exists in bicarbonate solutions of from 0.004 to 0.03 per cent (solutions I to IV); and in disodium phosphate solution of 0.016 and 0.024 per cent (solutions VII and VIII); 0.045 per cent bicarbonate (solution V) is too strongly alkaline, and 0.008 per cent phosphate (solution VI) is not sufficiently so. The addition of disodium phosphate to sodium bicarbonate in these concentrations (solutions IX to XI) raises the alkalinity but slightly, although it would of course in-

crease the buffer efficiency. Monosodium phosphate, 0.004 per cent, materially reduces the pH of 0.03 per cent bicarbonate (solution XII) by the elimination of CO_2. If this reaction is completed, the solution would finally contain 0.0275 per cent of bicarbonate and 0.0041 per cent of disodium phosphate. The Fleisch solution (XIII) is a special phosphate-bicarbonate formula which attains the desired pH of 7.5; but as has been stated, it becomes turbid on standing, indicating changes in its calcium and magnesium ions, and as these would be difficult to elucidate in so complex a solution, its use was discontinued.

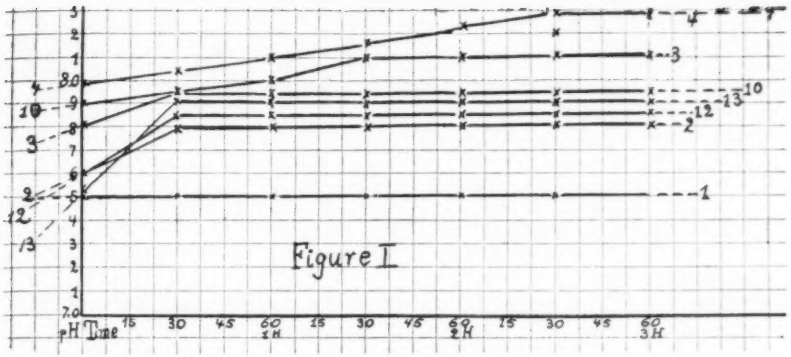
b. The effects of aeration on the pH under typical experimental conditions. Fairly brisk aeration (30 cc. of air per minute through 20 cc. of solution) for half an hour at 38° was found not to alter the pH materially, except with the solutions (XII and XIII) in which an excess of free CO_2 had been liberated by the addition of acid phosphate. Aside from these, it is therefore not necessary to consider changes through aeration in short experiments.

The effects of more prolonged aeration were investigated on the more promising of the solutions, namely, on the bicarbonate solutions of 0.004 to 0.03 per cent (I to IV); and on bicarbonate with the addition of disodium phosphate (X) and of monosodium phosphate solutions (XII). The Fleisch solution (XIII) was also tried. The bicarbonate free solutions, and those of alkalinity beyond pH 8 need not be considered for prolonged experiments, since they injure the functionation too severely. Four types of experimental arrangements were tested, employing the actual apparatus in current use for such work. The pH was again determined colorimetrically, on samples removed at intervals of 15 to 30 minutes. The results may be presented in the order of increasing aeration:

The first arrangement consisted in a 500 cc. perfusion bottle with Mariotte stopper, with the fluid discharging so as to aerate at the rate of six to twelve bubbles per minute, equivalent to 0.15 to 0.3 cc. of air per 100 cc. of solution. No significant change of pH occurred within two hours in any of the solutions, so that the aeration factor need not be considered for Mariotte stoppers.

The second arrangement represented the relatively slow aeration of a rather large bath; namely, the rectangular tank used for long pieces of intestine (von Oettingen, Sollmann and Ishikawa, 1928). In this, 5 liters of the solutions were aerated at the rate of 80 cc. of air per minute, equivalent to 1.6 cc. of air per 100 cc. The shape of the tank, however, renders the aeration much less effective than with an equal amount of air in a tall vessel. In this arrangement also the pH does not alter significantly within three hours in any of the solutions.

The third arrangement was that of the ordinary Magnus method, representing a small bath (50 cc.) and much more abundant aeration (60 cc.



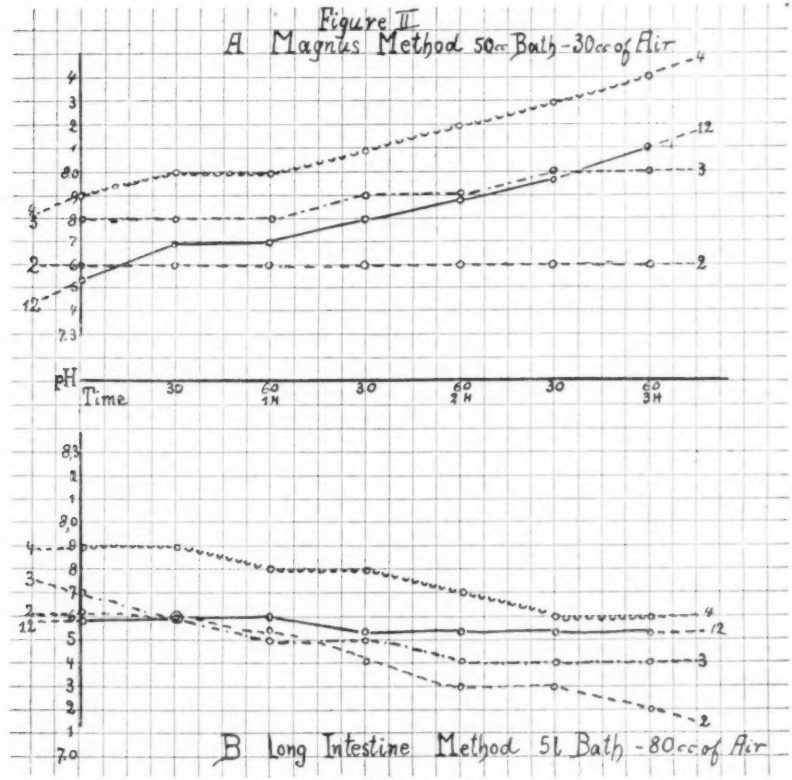


Fig. 1. The effect of aeration (60 cc. of air per 100 cc. of bath) on solutions I, II, III, IV, X, XII and XIII (see table 1) in a Magnus bath, but without intestines.

Fig. 2. The change of the pH in the presence of intestine using solutions II, III, IV and XII with the Magnus method (A) and the long intestine method (B).

of air per 100 cc. of solution). The results, as presented in figure 1, show that the bicarbonate solutions become very distinctly more alkaline, the degree of alkalinization increasing with the bicarbonate content of the pure bicarbonate solutions (I, II, III, IV) from practically nothing in 0.004 per cent bicarbonate; to a pH rise of 0.2 in 0.008 per cent and of 0.3 in 0.015 and 0.03 per cent bicarbonate; the time required practically to complete the alkalinization also increases with the bicarbonate content, from half an hour with 0.008, to 1½ hour with 0.015 and 2½ hours with 0.03 per cent bicarbonate.

The addition of disodium phosphate to the bicarbonate has a marked stabilizing effect against aeration: Whilst the alkalinity of solution III, containing 0.015 per cent of bicarbonate, rose from 7.8 to 8.1 = 0.3 pH, that of solution X, which contained 0.024 per cent of Na_2HPO_4 in addition to the bicarbonate, rose from 7.9 to 7.95 = 0.05 pH, i.e., within the limits of error of observation.

The addition of monosodium phosphate, on the other hand, hastens the alkalinization, although the end point is not higher: While in solution IV containing 0.03 per cent of bicarbonate, the pH rose in half an hour from 8.0 to 8.04 = 0.04; in solution XII, which contained 0.004 NaH_2PO_4 in addition, it rose from 7.6 to 7.8 = 0.2; and then remained at this level; whilst the alkalinity of the pure bicarbonate solution continued to increase to 8.3, a rise of 0.3 pH. The Fleisch solution (XIII) behaves essentially like the simple bicarbonate: biphosphate solution.

In working with small pieces of tissue, it may be necessary to deliver the air in very fine bubbles, so as to minimize the agitation of the solution (Siaulis and Sollmann, 1927). For the same ratio of air: solution this slows the rate of alkalinization; but the end points are practically the same.

c. Buffer efficiency in the presence of contracting intestine. The rabbits' intestine on which we were working seemed well suited for determining the buffer efficiency against the production of carbon dioxide and fixed acid by active tissues; not only because of its extensive use for experimentation, but also because the single organ may be made to perform two distinct types of movements, the pendulum and peristaltic; and this, with changes in the air supply, and in the ratio of tissue to solution, furnishes a variety of conditions that justify some generalizations. The buffers were restricted to the solutions most favorable to functional activity, namely, pure bicarbonate of 0.008, 0.015 and 0.030 per cent (solutions II, III and IV), and the 0.03 per cent bicarbonate with 0.004 per cent NaH_2PO_4 (solution XII); all added to the Ringer solution containing 0.1 per cent of dextrose. These were used at 38°C., with the perfused long intestine and with two arrangements of the Magnus method. With the 50 cc. Magnus method, the indicator was added directly to the bath; with the others, samples were removed at half-hour intervals for colorimetric comparison.

The relation between size of bath, quantity of intestine, and aeration are shown in table 2.

Figure 2 shows the course of the changes, and table 3 gives the initial pH and the changes at the end of three hours, with active intestines, and also for the Magnus arrangement without intestines (taken from the data of fig. 1). Since the long intestine tank does not alter its pH in the absence of intestines, it need not be tabulated. The data for the 400 cc. and 50 cc. Magnus baths were so nearly identical that only the latter is included in figure 2.

It will be seen from figure 2 and table 3 that the pH in the two arrangements takes a directly opposite course, the alkalinity decreasing with the long intestines and increasing in the Magnus arrangement. In the latter, the course is practically identical with that of the corresponding solutions without intestines (fig. 1). The aeration of the Magnus arrangement is therefore sufficient to remove as carbon dioxide all the acid produced by

TABLE 2

Aeration of bath with actively contracting intestines

CONDITIONS	LONG INTESTINE	MAGNUS, MODIFIED	MAGNUS, ORDINARY	RATIO LI : MM : MO
Solution in bath, cc.	5000	400	50	100 : 8 : 1
Aeration, cc. per minute per bath and per 100 cc. of solution	80 = 1.6 per 100 cc.	40 = 10 per 100 cc.	30 = 60 per 100 cc.	1 : 6¼ : 40 per 100 cc. of solution
Length of intestinal segment, cm.	100 = 2 per 100 cc.	2 = 0.5 per 100 cc.	2 = 4 per 100 cc.	1 : ¼ : 2 per 100 cc. of solution

the tissue, so that it does not noticeably affect the course of the pH. The alkalinization is due to the loss of carbon dioxide from the buffer itself. This increases with the richness of bicarbonate content, so that the 0.008 per cent bicarbonate is a more effective buffer for the Magnus arrangement than the 0.015 or 0.03 per cent; furthermore, the pH of the 0.008 per cent concentration is nearest to the optimal (7.5). The addition of the acid phosphate rather increases the pH change for the Magnus aeration, and from this standpoint at least would be less desirable than the pure bicarbonate buffer.

With the long intestines, as stated, the pH changes are toward the acid instead of the alkaline side; so that with this arrangement the aeration is not sufficient to remove the carbon dioxide produced directly by the tissue and indirectly by the action of the fixed acid on the bicarbonate. The difference between the two arrangements does not lie in the quantity

of active tissue, for in respect to the ratio of intestine to solution, the long intestine was intermediate between the two Magnus arrangements (table 2). Nor is it due to the type of activity, for the alkalinity of the large bath decreases in about the same degree in the absence of peristalsis, i.e., when the lumen of the intestines is not perfused, as will be shown in a later paper. The essential difference between the long intestine and the Magnus arrangement lies therefore not in the production of acid but in the removal of carbon dioxide, i.e., in the aeration, which is less efficient in the large tank, not only because of the smaller ratio of air, but because of the less effective agitation.

The increase of acidity with the long intestines is practically the same (0.3 pH) for the three bicarbonate solutions, so that their buffer efficiency

TABLE 3

Changes of pH at the end of three hours, with and without active intestines

BUFFER	pH AT START*	pH AT END OF THREE HOURS				CHANGES OF pH IN THREE HOURS			
		Long intestine*	400 cc. Magnus with intestines	50 cc. Magnus with intestines	50 cc. Magnus without intestines	Long intestines*	400 cc. Magnus with intestines	50 cc. Magnus with intestines	50 cc. Magnus without intestines
II = $NaHCO_3$ 0.008 per cent	7.6	7.2	7.6	7.6	7.8	−0.4	0	0	+0.2
III = $NaHCO_3$ 0.015 per cent	7.8	7.4	7.9	8.1	8.1	−0.4	+0.1	+0.3	+0.3
IV = $NaHCO_3$ 0.030 per cent	7.9	7.6	8.3	8.4	8.3	−0.3	+0.4	+0.5	+0.4
XII = $NaHCO_3$ 0.030 per cent + NaH_2PO_4 0.004 per cent	7.5	7.5	8.0	8.1	7.9	0	+0.5	+0.6	+0.3

* In the absence of intestines, the pH of the long intestine tank is not changed by three hours' aeration.

is equal; but since they buffer at different levels of pH, they are of very unequal value for functionation. If loss of alkali is unavoidable, it is better to start with a relatively high alkalinity; for not only is a moderate excess of alkali less deleterious to function than an equivalent deficiency, but if one starts with high alkalinity, the reaction becomes more and more favorable as the experiment proceeds, thus counterbalancing the natural deterioration of the excised organ; whilst if the experiment were started at the optimal pH, the unfavorable effects of the increasing alkali deficit would add themselves to the progressive deterioration due to the other unnatural conditions.

The most perfect buffering occurred by the addition of 0.004 per cent of monosodium phosphate to the 0.03 per cent bicarbonate in solution XII, due mainly to the liberation of an excess of the volatile carbon dioxide through the acid phosphate, which helps to balance the production of fixed acid by the intestine; and partly also to the stabilizing effect of the phosphate itself. Such a perfect balance as was attained in this arrangement, however, is merely a happy accident, which would not apply to other arrangements; for instance, with the more active aeration of the Magnus methods, the addition of the acid phosphate rather diminished the buffer efficiency.

In brief, 0.008 per cent of bicarbonate is the most efficient buffer for the Magnus arrangement; with less perfect aeration, the percentage of bicarbonate must be increased to 0.03 per cent, to keep the pH within the optimal range. This may be further stabilized by the addition of monosodium phosphate, but this would need to be determined for each arrangement. In any case, it is advisable to check colorimetrically the efficiency of any buffer for the particular arrangement that is used.

d. The maintenance of constant pH by the progressive addition of alkali or acid. Although the automatic control of the pH of the bath by suitable buffers is quite practical, it is possible with sodium bicarbonate only by adapting the quantity of the buffer to the type of aeration, and this entails rather strict limits both for the concentration of the bicarbonate ions and for the level of the pH that can be successfully maintained. This limitation is not serious for many types of work; but if the maintenance of a constant pH is critical, or if the pH and bicarbonate are to be adjusted independently of each other, continuous colorimetric control is practically indispensable, as has been pointed out by Michaelis and others. For this it is convenient to add the indicator directly to the bath in such dilution that it gives the same tint as the colorimetric control tubes; for instance, in the five liter tank, 0.5 cc. of a 0.02 per cent solution of the indicators is added per 100 cc. of Locke solution, to compare with the standard buffers which contained 0.5 cc. of 0.02 per cent of indicator per 10 cc. of solution. The tints are easily matched if they are viewed by reflected day light against the white enamel of the tank; with glass baths, white paper may be used as background. In either case, a set of standard buffer tubes is suspended over the bath.

When the color of the bath begins to change, it should be restored by the addition of alkali or acid; if the aeration is liberal, n/10 $NaHCO_3$ should be used as alkali; with limited aeration n/100 NaOH may be preferable; as acid, n/100 HCl seems preferable to phosphoric acid, since the latter introduces needless complication of the solution. With the Magnus method, the acid or alkali may be added from an ordinary burette; but for the large tank it is advisable to hasten the distribution of the solution by

attaching a right-angled tube to the burette, so that the solution may be spread over a wide arc by slowly rotating the burette.

CONCLUSIONS

The concentrations of sodium bicarbonate and phosphates which give the optimal range of pH to Locke solution were determined, as shown in table 1.

The increase of alkalinity by aeration is negligible for Mariotte bottles or larger tanks, even after two or three hours. With the free aeration and agitation that is used in Magnus baths, however, all the bicarbonate solutions become more alkaline; with 0.008 per cent bicarbonate, this is practically negligible. The degree of alkalinization, and the time required to reach a constant level, increase with the concentration of the bicarbonate. Na_2HPO_4 has a marked stabilizing effect, while NaH_2PO_4 hastens the alkalinization.

With the liberal aeration of the Magnus arrangement, the presence of the contracting intestines does not materially affect the aeration curve; but with the imperfect aeration of the larger tank, the pH takes the opposite direction, i.e., the alkalinity decreases. With the Magnus arrangement, 0.008 per cent sodium bicarbonate is a sufficient buffer and maintains excellent function of the intestines; with less aerated arrangements, it is impossible to maintain a constant pH by bicarbonate alone; but with 0.024 to 0.03 per cent of bicarbonate the pH remains within the optimal range. A constant pH may be attained by the addition of NaH_2PO_4 to the bicarbonate; but the amount would need to be varied according to the aeration. If a definite pH is essential, it is generally more simple to maintain it by the periodic addition of a bicarbonate solution (or hydrochloric acid), under colorimetric control.

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THE COMPARATIVE EFFECT OF EPINEPHRIN UPON BLOOD PRESSURE AND INTESTINAL MOTILITY IN THE DOG

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In 1912 Hoskins and McClure, working on dogs anesthetized with ether reported that "intestinal depression is caused by injections of epinephrin decidedly smaller than those requisite to cause increase of blood pressure." More recently Durant (1925) corroborated this finding on cats and dogs anesthetized with ether and urethane. He reported "that with cats and dogs blood pressure could be raised and maintained at a pressor level only at the expense of gastro-intestinal motility." In rabbits, however, anesthetized presumably in the same way, he reported "that in these animals the blood pressure can be raised and maintained slightly above normal by injections of adrenalin that are without significant effects upon the intestine."

It is generally conceded that anesthetics depress the motility of the gastro-intestinal tract to a varying degree. This has been shown clearly in the recent work of Miller (1926). It seems reasonable to suppose that the depressant effect of epinephrin would be more readily produced in the anesthetized than in the unanesthetized animal. Similarly it has been shown that vascular responses to epinephrin in small doses are less readily produced in the anesthetized than in the unanesthetized animal. It is possible if not probable, therefore, that a study of the comparative effect of epinephrin upon blood pressure and intestinal motility would yield directly opposite results depending upon whether or not general anesthesia is used.

The experiments were done in various ways and will be illustrated by the following protocols. In all instances adult dogs in good physical condition were used. Intestinal motility was recorded by means of an air balloon and piston recorder. Blood pressure tracings were taken from the carotid artery. Solutions of epinephrin in distilled water, freshly prepared from pure crystalline adrenalin supplied by Parke, Davis & Co. were used. Injections were made into the saphenous or femoral vein by means of a Woodyatt pump.

Group I. The experiments in this group are illustrated by the following protocol.

Experiment 18. Female dog. Weight, 14.5 kilos.

November 5, 1927. Thiery fistula prepared from section of the jejunum under ether anesthesia.

November 15, 1927. Carotid artery transplanted externally to the skin, under ether anesthesia.

November 16, 1927. Dog placed on table. Cannula inserted into carotid transplant. Balloon placed in Thiery fistula. Femoral vein exposed and cannulated under 3.0 cc. of 1 per cent procain. As the animal was quite restless 15 mgm. of morphine sulphate were injected subcutaneously. Graphic records showed a considerable rise in blood pressure is obtained before intestinal paralysis occurs. Two other experiments performed in a similar manner gave like results.

Group II. As the experiments in group I are open to the objection that the procain used may have sensitized the animal to the vascular effect of epinephrin more than to the intestinal effect, experiments in this group were performed without local anesthesia in the following manner.

Experiment 22. Female dog. Weight, 16.1 kilos.

November 17, 1927. Thiery fistula prepared from jejunum under ether anesthesia.

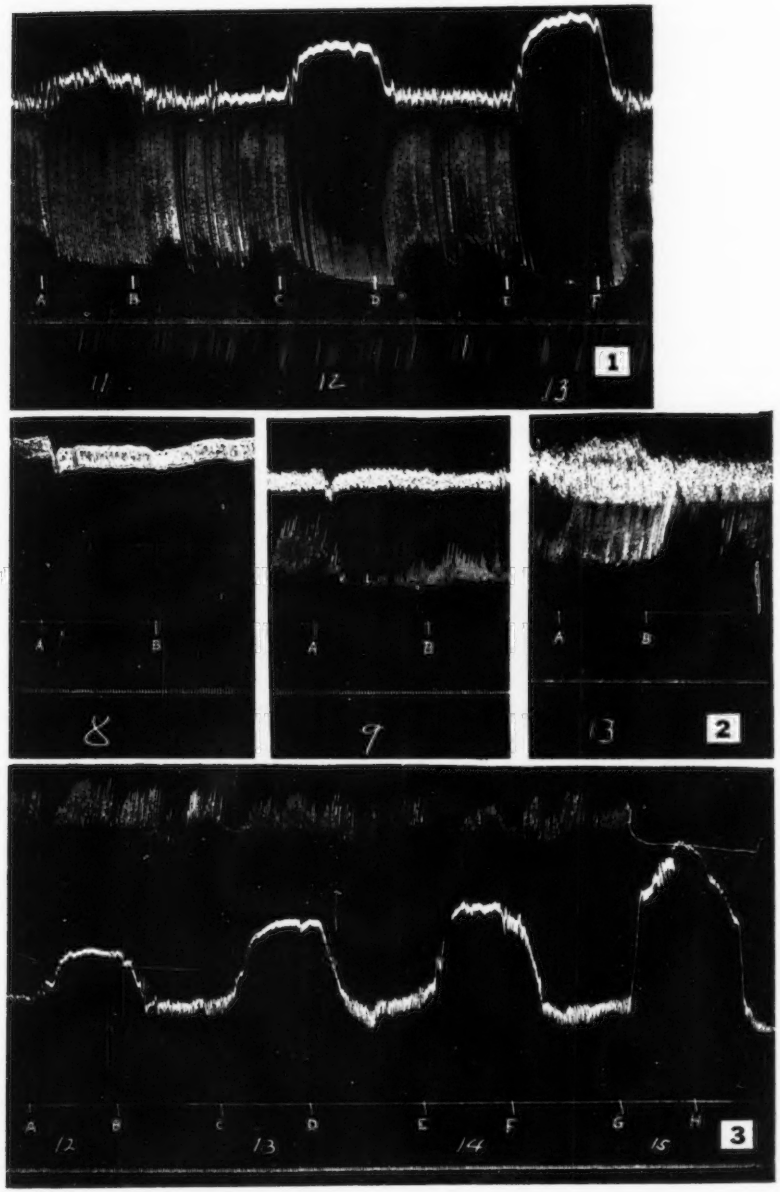
November 27, 1927. Ether anesthesia. Carotid artery transplanted externally to the skin. Spinal cord transected below Thoracic XII so that the femoral vein could be exposed and cannulated on the succeeding day without disturbing the animal.

November 28, 1927. Dog placed on table. Cannulae inserted into femoral vein and carotid transplant. Balloon placed in Thiery fistula. Dog somewhat restless, so that intestinal motility was uncertain. Fifteen milligrams of morphine sulphate injected subcutaneously. Following this injection the dog went quietly to sleep. At this time the record shown in figure 1 was obtained.

Fig. 1. Experiment 22 (11), (12) and (13). Female dog, 16.1 kilos. Jejunal fistula 10 days, carotid transplanted 24 hours, spinal cord transected below Thoracic XII for 24 hours. Morphine sulphate 15 mgm. prior to experiment. *A* to *B*, 1.0 cc. of 1 to 1,000,000 adrenalin per kilo per minute for 4 minutes; *C* to *D*, 2.0 cc. of 1 to 1,000,000 adrenalin per kilo per minute for 4 minutes; *E* to *F*, 2.7 cc. of 1 to 1,000,000 adrenalin per kilo per minute for 4 minutes.

Fig. 2. Experiment 19 (8), (9) and (13). Male dog, 24.3 kilos. 8—Ether anesthesia. 9—30 minutes after ether stopped. 13—2 hours after ether stopped. Upper trace—carotid blood pressure; second trace—contractions of jejunum; third trace—signal line; bottom trace—time in 6 seconds *A* to *B* in each instance, 1.4 cc. of 1 to 1,000,000 adrenalin per kilo per minute for 4 minutes.

Fig. 3. Experiment 16 (12), (13), (14) and (15). Male dog, 14.5 kilos. No drugs. Top tracing—contractions jejunal fistula; second tracing—carotid blood pressure; third tracing—signal line; bottom tracing—time in 6 seconds and base line of blood pressure. *A* to *B*, 0.56 cc. 1 to 1,000,000 adrenalin per kilo per minute for 4 minutes; *C* to *D*, 0.90 cc. 1 to 1,000,000 adrenalin per kilo per minute for 4 minutes; *E* to *F*, 1.34 cc. 1 to 1,000,000 adrenalin per kilo per minute for 4 minutes; *G* to *H*, 3.68 cc. 1 to 1,000,000 adrenalin per kilo per minute for 3.5 minutes.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As is clearly demonstrated in the tracing a considerable rise of blood pressure occurs before intestinal paralysis obtains, even when the injection is prolonged for a period of ten minutes. Similar results were obtained in three other experiments performed in the same way and also in a fourth animal to whom morphine and atropine were administered.

Group III. Since the experiments performed in groups I and II had been with animals from which intestinal motility was recorded by means of a Thiery fistula, it seemed advisable to secure a series in which the contractions of the intact intestine might be obtained. Likewise since the observations in the literature relative to the comparative effect of epinephrin upon blood pressure and intestinal motility under ether anesthesia did not coincide with the results as shown in the preceding groups, it became necessary to repeat the observations on animals under general anesthesia. The animals were then allowed to recover under morphine or morphine and atropine.

A protocol of one experiment in this group follows.

Experiment 19. Male dog. Weight, 24.3 kilos.

November 17, 1927. Ether anesthesia was administered. Tracheal cannula was inserted and attached to a Wolf ether bottle. Carotid artery and femoral vein cannulated. Abdomen opened and air balloon introduced into duodenum through a small incision. Abdomen closed. Fifteen milligrams of morphine sulphate were injected subcutaneously, and the ether stopped. No intestinal motility was apparent until the ether had been removed for ten minutes. The tracing shown in figure 2 was obtained at this time.

As is seen in the middle tracing of the figure, intestinal inhibition can be obtained with epinephrin when no rise in blood pressure is apparent at a certain stage in the recovery from ether. On further recovery from ether, however, definite rises in blood pressure can be obtained without intestinal inhibition.

Four additional experiments of the same nature have been done with similar results. The experiment has also been repeated in the reverse order (e.g., by recording blood pressure and intestinal motility before anesthesia and after a light anesthesia with ether has been obtained).

Group IV. The experiments under groups I, II and III are open to the objection that morphine was used which, as has been shown by Plant and Miller (1926) and confirmed by us in these experiments, increases the tone and activity of the small intestine. It is possible that the threshold of response to epinephrin has been raised by the morphine. Several experiments were therefore done as in group III, except that animals were allowed to recover from the ether under paraldehyde. In each instance we were able to show a rise in blood pressure without intestinal inhibition.

In one dog under trional, one under barbital and one under amytal intestinal motility was very weak and inconstant. In all three, inhibition of

the intestine appeared before definite rises in blood pressure could be obtained.

In one dog in which the experiment was done as in group III, except that the animal was stunned by a severe blow over the head, definite rises in blood pressure could be obtained without any intestinal inhibition.

Group V. The experiments in groups I, II, III and IV are open to various theoretical objections because of the use of anesthetics, drugs or trauma. It is desirable therefore to repeat the observations on physiologically normal animals. Dogs that were trained to lie quietly on the table without the aid of morphine or any other depressants were used. The experiments were performed as in groups I and II except that no local anesthetics or morphine were used. In two animals the injections were made by means of a needle inserted in the saphenous vein and in two others by exposure of the femoral vein after a previous spinal cord transection below Thoracic XII.

In all four experiments definite rises in blood pressure could be obtained without intestinal inhibition. See figure 3.

It seems clear from these results that pressor effects can be obtained with epinephrin without the price of intestinal paralysis. It is apparent that ether tends to wipe out intestinal motility. It also decreases the pressor response to epinephrin. As the depth of the anesthetic varies the comparative responsiveness of the vascular system and intestine to epinephrin likewise varies. As the animal is allowed to recover from ether the blood pressure is more likely to be raised by small doses of epinephrin and the intestine less likely to be inhibited.

The observations of Plant and Miller that morphine stimulates many phases of muscular activity of the small intestine in dogs have been repeatedly confirmed. Epinephrin in sufficient dosage would still produce inhibition after morphine, but whether or not the threshold for inhibition has been changed is not clear. From the experiments in group V it is certain that the threshold has not been changed sufficiently to alter the ratio between the sensitivity of the vascular and intestinal response.

One of the objections to the tonus theory of medulliadrenal function has been that blood pressure effects could not be obtained except at the price of intestinal paralysis. It is not our intention to discuss this theory except to state that in view of the findings reported in this paper, this objection can no longer be maintained.

SUMMARY

1. The simultaneous effects of continuous injections of epinephrin on blood pressure and intestinal motility were recorded in dogs during and after anesthesia with ether, under morphine, under paraldehyde, and in the absence of drugs and anesthetics.

2. It was found that with a certain amount of ether and in three instances with barbital, trional and amytal the blood pressure could be raised only at the expense of intestinal motility.

3. In normal dogs and in dogs under morphine, paraldehyde or after stunning, the opposite relationship obtains. In these conditions considerable rises in blood pressure can be obtained without intestinal inhibition.

The authors acknowledge with thanks the assistance of R. S. Hardwick in the performance of the experiments.

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STUDIES IN EXHAUSTION DUE TO LACK OF SLEEP

V. Effect on the Thyroid and Adrenal Glands with Special Reference to Mitochondria

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In our previous articles on "Exhaustion due to lack of sleep" (1927) we have cited the importance of an accurate knowledge of the symptomatology and structural changes in the tissues resulting from functional disorders of the central nervous system. We have described an experimental method for producing exhaustion from lack of sleep and the resulting morphological changes in the cells of the spinal cord and medulla.

That changes in the endocrine glands are often associated with functional disorders of the central nervous system is a well-known fact as is shown in exophthalmic goiter with an increased nervous excitability and in cretinism with its decreased nervous activity. Since the abnormal functioning of the thyroid gland for example is usually accompanied by functional disorders of the central nervous system it would be of extreme interest and importance to know whether exhaustion of the central nervous system is accompanied by morphological change in the thyroid and adrenal glands.

As far as we have been able to find, no histological studies have been made of the endocrine glands to determine the effect of nervous exhaustion due to lack of sleep.

Early cytological changes are not easily detected in the endocrine glands. Recent researches indicate that the mitochondria of the cell are very sensitive to functional changes or pathological conditions. Thus Altmann (1894) first showed that the shape and number of specifically stained gramules (bioblasts) varied with the functional state of the gland cells. (He used the term "bioblast" for all granules. The term mitochondria which Benda (1897) introduced refers to those granules which react specifically to Altmann's acid fuchsin aniline solution.) He noticed that in resting cells these mitochondria are numerous and often appear filamentous while during activity the filaments disappear and the number are reduced, or are absent entirely after prolonged activity. Scott (1916) working on

the pancreas in cases of phosphorus poisoning and Nicholson (1923) working on the thyroid gland have shown marked mitochondrial changes where nuclear change was hardly apparent. For other references relative to the presence and function of mitochondria see Cowdry (1924).

In our study of the thyroid in exhausted rabbits we have followed this clue and prepared the thyroids for the study of mitochondrial changes. Unfortunately none of the adrenal glands were prepared for the study of mitochondria.

THE THYROID GLAND. The thyroid glands upon which the following observations are based were taken from the normal and exhausted rabbits described in our previous articles on "Studies in Exhaustion due to Lack of Sleep" (1927). The thyroids were removed immediately upon the death of the animals and fixed in an acetic-osmic-bichromate solution consisting of:

2.5 per cent Potassium bichromate	9 cc.
4.0 per cent Osmic acid	2 cc.
Glacial acetic acid	1 drop

The tissues were imbedded in paraffin and sectioned serially at 3 to 4 micra and stained in Altmann's acid fuchsin aniline solution and counterstained in a 1 per cent solution of Methyl green. Great care was taken to standardize our technique in order to obtain constant results.

OBSERVATIONS. *A. The normal thyroid.* These observations were made on the thyroids from our control rabbits. The nuclei lie in the central or slightly basal part of the cells. They are round or slightly oval depending upon the shape of the cells and have an average diameter of about 6 micra. The nuclear membrane is well defined while the content usually takes a diffuse basic stain. The chromatin is quite uniformly distributed. The chromatin net knots and nucleoli are visible but not especially prominent. The nucleoli are from one to four in number.

The cytoplasm takes a general greenish stain while the mitochondria are bright red. The mitochondria, in the majority of cases, are round, occasionally rod shaped and are usually thickly scattered throughout the cytoplasm at the basal end of the cells. In some cases the free border of

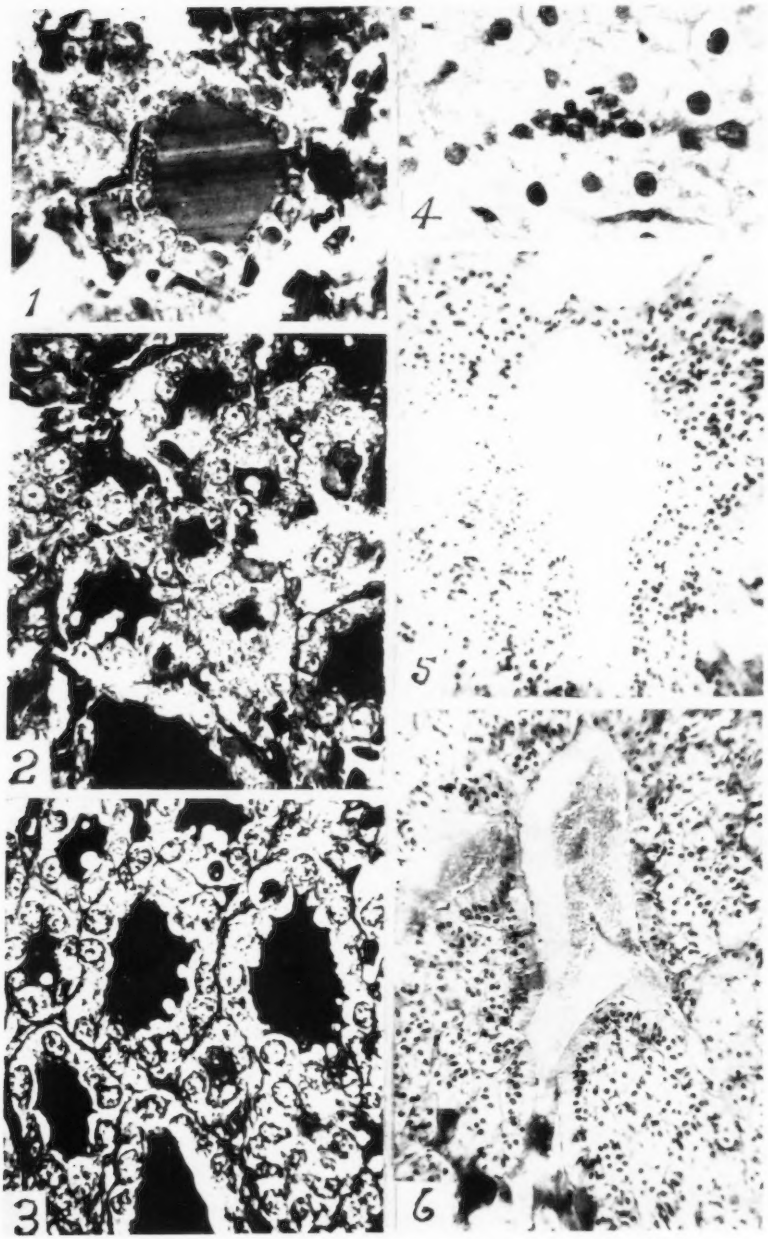
Figs. 1 and 2. Photomicrographs of normal thyroids showing numerous mitochondria. The nuclei in figure 1 are diffusely chromatic and in figure 2 they are more acromatic.

Fig. 3. A section of a thyroid from an exhausted rabbit showing the swollen achromatic nuclei and the marked reduction of mitochondria.

Fig. 4. Nest of eosinophiles in the adrenal cortex of an exhausted rabbit.

Fig. 5. Sinuses of adrenal medulla of a normal rabbit.

Fig. 6. Sinuses of adrenal medulla of an exhausted rabbit showing content of colloid and cellular debris.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the cells appears to be ruptured and in such cases mitochondria are found within the colloid. Rod shaped mitochondria when present were always short.

B. The thyroid glands of exhausted rabbits. The nuclei are swollen and measure from 7 to 8 micra in diameter. The chromatin is not diffusely distributed as in the normal nucleus but is present in small masses lying close to the nuclear membrane while the nucleoli retain their normal position. The rest of the nucleus does not take the stain. In the normal thyroid one occasionally sees nuclei more or less achromatic but not as markedly so as in the exhausted thyroid. Figures 1 and 2 are photomicrographs from normal thyroids. Figure 1 shows the diffusely staining nuclei of the type commonly found and figure 2 represents nuclei which are more achromatic but which are rarely found in the normal thyroid. Figure 3 represents the typical achromatic swollen nuclei found in the exhausted thyroids of the rabbits.

In the cytoplasm the most conspicuous change from the normal is the marked decrease of mitochondria as can be seen by comparing figures 1 and 2 with figure 3. The cytoplasm also has a clearer appearance and the free border of the cells is more or less irregular.

Discussion. If one may draw the conclusion from the swollen and dechromatized nucleus and the depleted cytoplasm, that the cells have passed through a hyperactive state, then the decrease of mitochondria would indicate that they are associated and used up in the production of secretion. Altmann (1894) observed this decrease of the specifically acid fuschin stained granules following hyperactivity of gland cells. He concluded that they are metabolic centers around which the secretion droplets are built up. His plates III, XXIV, XXV, XXVI and XXX illustrate this building up of fat or secretion droplets around these granules. That mitochondria are related with cell activity and the production of cell products has been described by many recent writers but apparently without giving credit to Altmann for first presenting this interpretation. For references and discussion of mitochondria see Cowdry (1924).

SUMMARY

1. In the thyroid gland of exhausted rabbits the number of mitochondria is markedly decreased.

2. The nuclei are swollen and the decreased chromatin is clustered along the nuclear membrane.

The adrenal glands. The adrenal glands were removed immediately upon the death of the animals and fixed in Orth's fluid. The tissues were imbedded in paraffin, sectioned serially and stained with hematoxylin and eosin.

RESULTS. The adrenal glands of fourteen exhausted rabbits were studied. It is not easy to detect slight pathological changes in the adrenal because the histological pictures of supposedly normal rabbit adrenals vary greatly. Not only does the organization vary but the cell content may be highly granular in one and vacuolated in another rabbit.

In our exhaust adrenals, however, we noted certain pictures which we interpreted as abnormal.

1. The cells of the cortex in 13 of the 14 adrenals were markedly vacuolated and many of the nuclei took the eosin stain while others were pyknotic with irregular outlines. Crile (1921) describes similar changes after 100 hours of loss of sleep. Frabfield and Squire (1921) noted pyknotic nuclei and vacuolization of cells with lessened staining power of adrenals of rabbits exposed to x-ray irradiation.

2. The adrenal glands of five of the fourteen rabbits had nests of eosinophiles in the sinuses of the cortex as is shown in figure 4. This percentage is not sufficiently high to permit the conclusion that this condition is due to exhaustion.

3. In eleven of the fourteen cases we found marked congestion of the medullary sinuses and vessels with either a colloidal substance or cellular debris. Compare figures 5 and 6. This was one of the most outstanding differences from the control adrenals.

4. In the region where the medulla joins the cortex most of the cells are large and have an embryonic appearance while between them are degenerating cells.

SUMMARY

We do not feel that from our study so far we are able to draw any conclusion regarding relation of the adrenal glands to nervous activity. Further researches are necessary. We are only able to give the following variations from the normal adrenal.

1. Increased vacuolation of the cytoplasm with pyknotic nuclei.

2. Scattered patches of eosinophiles in the cortical sinuses. (Shown by 36 per cent of the rabbits.)

3. A marked congestion of the sinuses and vessels of the medulla with a colloid-like material and cellular debris.

4. In the medullary-cortical zone the cells are large and have an embryonic appearance and between them degenerating cells are found.

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THE EFFECT OF SOME DIURETICS ON THE UREA EXCRETING CAPACITY OF THE KIDNEY[1]

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Although under ordinary conditions the rate of urea excretion is influenced by many factors (1), it has been shown (2) that under certain special conditions the rate of urea excretion varies within narrow limits in direct proportion with the blood urea concentration. Under these conditions the ratio $\frac{\text{urine urea rate}}{\text{blood urea concentration}}$ for a given individual is therefore a constant. By the addition of some new factor to these special conditions the effect on the rate of urea excretion can be measured (3) by the deviation of the ratio from its standard value. In the present study the effect of various diuretics on this ratio has been determined. All of the experiments were carried out on a single subject (Pol), a young adult male weighing 75 kilograms and in excellent health.

The "standard" conditions under which the ratio was determined have been described in detail elsewhere. They are designed to produce a high degree of renal activity. Briefly they may be outlined as follows: No food is taken during the experimental period nor for 12 hours preceding. At the beginning of the first hour (usually 6 a.m.) the subject drinks 20 cc. water per kilogram in which is dissolved 0.25 gram urea per kilogram of body weight. Ten cubic centimeters water per kilogram are taken at the beginning of each hour thereafter until the end of the experiment. Commencing with the fourth hour, hourly urine specimens are collected. These are timed to within 30 seconds and special precautions are taken to insure exact and complete voiding. It is essential that the subject be standing or using a commode when the specimen is taken. A specimen of venous blood is drawn at exactly the middle of each hourly urine period. The urea content of the blood and urine specimens is determined by methods (4) which have proven to be especially adapted to such a study.

The observations which were obtained under these conditions comprise table 1 and serve as a control. The average ratio is 50.6. The probable

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TABLE 1

The ratio: $\frac{\text{Urea in 1 hour's urine}}{\text{Urea in 100 cc. of blood}}$ *in the subject Pol. under standard conditions*

URINE VOLUME	URINE UREA	BLOOD UREA	RATIO: $\frac{\text{URINE UREA}}{\text{BLOOD UREA}}$
cc. per hr.	*mgm. per hour*	*mgm. per 100 cc.*	
455	2840	58.8	48.3
465	2790	53.8	51.9
390	2773	54.5	50.9
425	2690	50.0	53.8
450	2670	50.8	52.6
540	2610	48.5	53.8
515	2559	50.2	51.0
380	2542	49.4	51.5
470	2540	48.1	52.8
660	2531	46.5	54.4
330	2432	50.2	48.5
445	2340	49.0	47.7
540	2334	48.1	48.5
365	2334	44.6	52.4
470	2328	44.0	52.9
525	2310	45.6	50.6
178	2304	48.1	47.9
500	2290	46.6	49.1
795	2280	44.4	51.4
425	2270	46.3	49.0
580	2263	44.1	51.4
480	2250	45.6	49.3
485	2235	42.1	53.1
630	2192	45.9	47.8
565	2170	41.8	51.9
560	2144	42.1	51.0
700	2140	39.5	54.2
480	2117	44.4	47.7
445	2076	39.9	52.0
425	2072	41.5	49.9
580	2029	40.2	50.5
475	2007	42.0	47.8
470	2003	41.9	47.8
560	1950	35.4	55.1
615	1865	39.0	47.8
600	1704	35.8	47.6
380	1700	34.0	49.7
440	1599	31.7	50.4
595	1554	32.1	48.4
Average			50.6
Standard deviation			2.22
Variability			4.38 per cent

error is only ±0.231 so that a comparatively small experimental change becomes significant. Most of the experimental changes produced in the ratio pass far beyond the range of variation of the control series. In the figure which has been used to demonstrate the effect of different diuretics on the ratio, the darker shaded area on either side of the mean covers a range four times the probable error. This range includes practically all the results which might have been obtained by numerous repetitions of the experiment.

Each experiment was carried out in exactly the same manner as in the case of the normal observations described above with the addition that an appropriate dose of the diuretic under consideration was taken after a control hour, that is, at the commencement of the second hour of observation. A diuretic we understand as a substance the ingestion of which produces an increase in the renal excretion of water. This property of a diuretic drug is per se without effect in the present study. Urine volume has been demonstrated (5) to bear no relation to the rate of urea excretion when the functional activity of the kidney is maintained at the same level for every measurement of urine volume. If varying quantities of fluid are ingested from time to time a corresponding variation in the degree of renal activity for water and urea excretion results and the rate of urea excretion appears (6) to be regulated by the urine volume. This does not influence our results for all measurements were made under conditions designed to insure the maximum degree of renal activity.

1. The xanthine diuretics. The standard conditions were maintained while the first measurement was being made. The drug was given in each case at the end of this hour. Caffeine was administered in the form of the citrate, theophyllin as such and theobromine as the sodium salicylate compound. All three of these drugs increased the rate of water excretion even at the high level at which it was measured but the volume changes bear no constant relation to the changes in the ratio.

Coffee has already been shown to cause an increase in the rate of urea excretion and at this time the caffeine was found to be responsible. Figure 1a shows the effect of varying doses of the caffein diuretic. The ingestion of 4 mgm. per kilogram of body weight is without any certain effect on the ratio although the urine volume was increased far more than with larger doses. Both 10 mgm. and 16 mgm. of caffeine per kilogram caused a striking increase in the ratio which lasted throughout the three hours during which observations were made. The larger amount had no greater effect than the 10 mgm. dose.

The ratio is practically unaltered by the ingestion of 4 mgm. theobromine per kilo as shown in figure 1b while larger doses (10 and 16 mgm. per kilo) were aboutequally efficacious in causing a definite increase in the ratio.

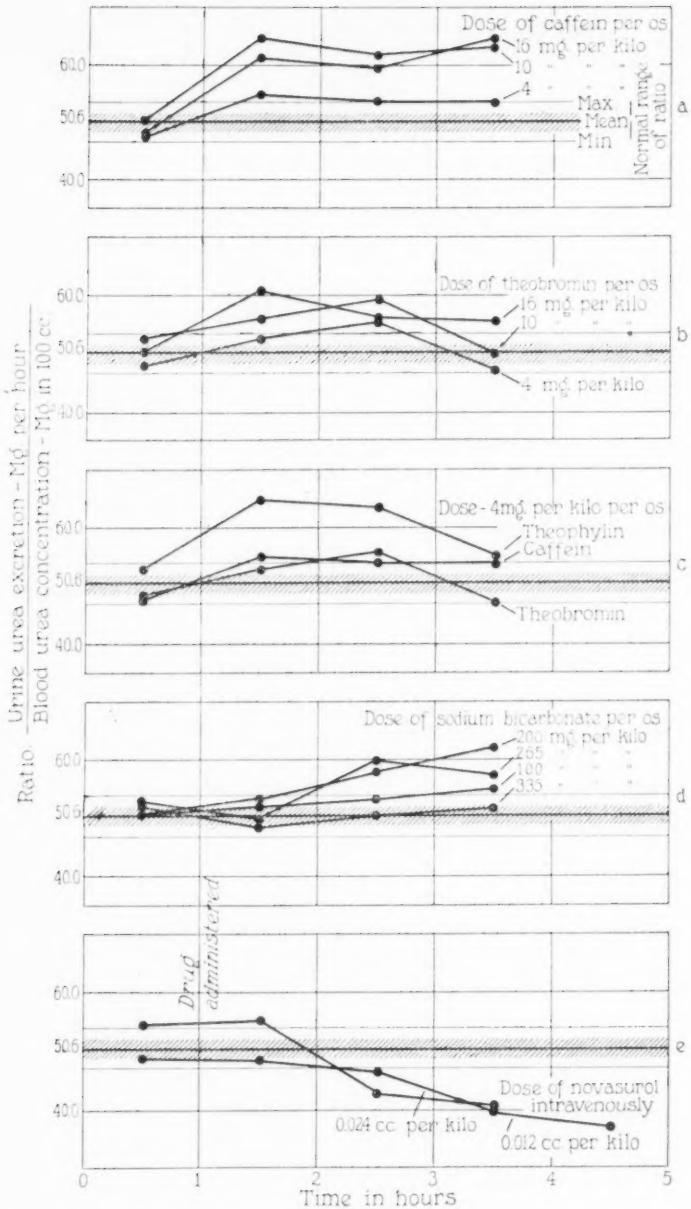

Fig. 1. Graphic representation of the effects of various diuretics upon the rate of urea excretion as measured by the ratio: $\frac{\text{Urine urea rate}}{\text{Blood urea concentration}}$ before and subsequent to their administration.

Theophyllin was given in only a single dose—4 mgm. per kilogram. Its administration resulted in a higher ratio than any dose of the two other drugs. In figure 1c the effects of an equivalent amount (4 mgm. per kilogram) of each of the three diuretics on the ratio have been compared. It is generally conceded (7) that theobromine is superior to caffeine as a diuretic. This is true only when the former is given in larger amounts. Dose for dose caffeine is the stronger diuretic. The same relation holds in their effect on the rate of urea excretion. The superiority of theophyllin over these two drugs is genuine. Likewise it has the most marked effect on the ratio.

TABLE 2

Effect of hydrochloric acid

Standard conditions until second hour when the diuretic was taken

TIME	RATIO: URINE UREA / BLOOD UREA	URINE UREA	BLOOD UREA	URINE VOLUME
Hydrochloric acid—8 mgm. per kilo (175 cc. N/10 HCl)				
		mgm. per hour	*mgm. per hour*	*mgm. per hour*
8:30– 9:30 a.m.	51.0	2559	50.2	515
9:30–10:30 a.m.	52.1	2482	47.7	370
10:30–11:30 a.m.	**57.5**	2452	42.7	855
11:30–12:30 p.m.	49.8	2000	40.2	595
Calcium chloride—50 mgm. per kilo (3.75 grams $CaCl_2$)				
8:30– 9:30 a.m.	50.9	2773	54.5	390
9:30–10:30 a.m.	**55.1**	2618	47.5	318
10:30–11:30 a.m.	**59.5**	2710	45.6	502
11:30–12:30 p.m.	54.6	2291	42.0	570
Calcium chloride—100 mgm. per kilo (7.5 grams $CaCl_2$)				
8:30– 9:30 a.m.	48.3	2840	58.8	455
9:30–10:30 a.m.	47.4	2533	53.5	190
10:30–11:30 a.m.	**53.6**	2650	49.5	465
11:30–12:30 p.m.	49.4	2269	45.9	630

2. Acids. It is well known that mineral acids have a strong diuretic action. Hydrochloric acid in the amount of 2.33 cc. of a 0.1 N solution per kilogram of body weight caused a definite increase (table 2) in the rate of urea excretion. About 40 per cent of the chlorine in calcium chloride has been found (8) to act as an acid when this salt is ingested. Calculated on this basis an approximately equivalent amount of hydrochloric acid given in the form of calcium chloride (50 mgm. per kilo) gave about the same increase in the ratio as resulted from the straight acid solution. Double

this quantity of the calcium salt (100 mgm. per kilo) was, on the other hand, without any effect on the ratio, probably because of its poor absorption and the passage of fluid into the intestine. We may assume the latter from what we know of the usual behavior of this salt and from the extreme decrease in urine volume which took place in the present experiment.

The effect of a variation in the acid base equilibrium toward the acid side on the rate of urea excretion was determined by Nagayama (9) in experiments on rabbits. He observed a distinct decrease in the urea ratio after giving acid phosphate while a neutral mixture of acid and alkaline phosphate had no effect. Since the only essential difference in the condition of these experiments lay in the fact that after the neutral phosphate mixture the acid base equilibrium remained unchanged, whereas after acid phosphate there was a shift toward the acid side, he concluded that the decrease in the alkalinity of the plasma induced by an increase in the amount of acid phosphate within the body, decreased the urea excreting activity of the kidney. At first glance his results seem to be at variance with ours. This is, however, not necessarily the case. In the first place enormous doses of the phosphate in relation to body weight were used. When we gave small amounts of calcium chloride a definite increase in the ratio took place. In larger dosage, although there was no positive decrease the ratio was certainly not increased. This second result was presumably due to withdrawal of water into the intestine by the salt, a happening which might just as easily have affected Nagayama's phosphate results. Then it is probable that the effects on the kidney of acid given in the form of the free acid and as acid phosphate are very different and cannot be considered together. The free acid increases the excretion of ammonia and fixed base while acid phosphate is without effect on ammonia excretion and changes the excretion of fixed base very little. Sodium and phosphate are excreted unchanged whereas hydrochloric acid must first combine with a base.

3. Alkali. The results of the ingestion of various dosages of sodium bicarbonate have been compared in figure 1d. The salt in the dosage of 0.1 gram per kilo had no certain effect on the ratio. Double this amount (0.2 gram per kilo) caused a distinct increase in the rate of urea excretion commencing an hour after its administration. A larger dose (0.265 gram per kilo) had no greater effect and a still larger dose (0.335 gram per kilo) had no effect whatever on the ratio. When the experiments are examined the cause of the latter is evident. As the amount of sodium bicarbonate given in each experiment was increased the urine volumes decreased. In the last experiment they were the lowest and in this experiment the large dose of alkali caused a marked catharsis which began in the middle of the second hour and lasted throughout the experiment. This loss of water

into the intestine removed so much fluid from the kidney that a decrease in the degree of renal activity resulted. Although this did not bring the ratio below the normal level, the depression offset the effect of the alkali which is evident with a smaller dose of the salt. In this connection we will again refer to the experiments carried on in this laboratory by Nagayama (9). He found that the administration of amounts of sodium bicarbonate which markedly increased the alkalinity of the plasma was accompanied by a slight decrease in the urea excreting activity of the kidney. The rabbits in his experiments received an extremely large dose (1.5 gram per kilo) of bicarbonate and the effect of it in the rabbit is probably comparable to the 0.335 gram per kilo in man. One may then conclude that sodium bicarbonate causes an increase in the rate of urea excretion, when given in such dosage that extra-renal effects do not predominate.

4. Novasurol. Since Saxl and Heilig (10) showed the efficacy of the organic mercury compound novasurol (merbaphen) in cardiac dropsy, the drug has proven useful as a diuretic in hepatic edema and in some cases of edema associated with renal disease. In normal individuals the diuretic has been shown (11), (12) uniformly to increase the excretion of chlorides, although diuresis does not always occur. There are no data available concerning its effect on the rats of urea excretion.

In the two experiments reported here (fig. 1e) therapeutic doses of the drug were given intravenously. The commercial solution was used in the dosage of 0.012 cc. and 0.24 cc. per kilogram of body weight. The urine was free of albumin and the excretion of chlorides was increased as usual. The interesting result was a marked depression of the rate of urea excretion which followed the drug. It might be argued that this would be expected from mercury. However, there was no evidence of toxicity and a therapeutic dose was used. Even mercuric chloride when given in sub-toxic doses increases (13) the ratio or leaves it unchanged.

SUMMARY

All of the xanthine diuretics increase the urea excreting capacity of the kidney. Theobromine is the least powerful, caffeine more so while theophyllin has by far the strongest effect.

Hydrochloric acid and sodium bicarbonate given by mouth each increase the urea excreting capacity of the kidney.

The intravenous injection of a therapeutic dose of novasurol is followed by a marked depression in the urea excreting capacity of the kidney.

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SPONTANEOUS DECIDUOMATA IN PSEUDOPREGNANCY WITH LOW VITAMIN E[1]

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Twenty years ago Leo Loeb[2] showed that a remarkable reaction ensues in the endometrium when an injury is inflicted upon it or a foreign body is placed within it at a definite time in the ovarian cycle of the guinea pig. The reaction consists in a local deciduomatous transformation of the connective tissue cells of the tunica propia, so extensive as to produce distinct uterine tumors, which thus consist of maternal placental tissue without the possibility of fetal elements being present. Loeb's reaction was subsequently shown to be possible in a considerable number of other mammals, though it ordinarily cannot be provoked at any time in the very short ovarian cycle of the rat or mouse, as was shown by Long and Evans in 1920.[3] The latter authors, however, clearly demonstrated that typical tumors of this sort can be provoked in the rat in cases where the interoestrous interval is prolonged through prolonged function of the corpora lutea—as is the case, for instance, in lactation or in pseudopregnancy, a condition which they induced by stimulation of the cervical canal or by permitting copulation with a vasectomized male.

In all of the experiments cited, the introduced foreign body—the stimulating agent—consisted of silk thread, care being taken to see that the thread pierced the endometrium and in fact reached the uterine lumen. In the present communication we desire to record the remarkable fact that these deciduomatous tumors need not be provoked by operative interference with the uterus, but may be spontaneously formed under conditions as yet not wholly clear and that the tendency to their formation is very greatly increased by dietary regimens lacking fat soluble vitamin E. Our attention was first called to the possibility of the spontaneous occurrence

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and from the Bureau of Dairying of the United States Department of Agriculture. These funds have been generously augmented by the Board of Research and by the College of Agriculture of this institution.

[2] Loeb, L. The production of deciduomata and the relation between the ovaries and the formation of the decidua. (Journ. Amer. Med. Assoc., 1, June, 1908.)

[3] Long, J. A. and H. M. Evans. The oestrous cycle in the rat (Memoirs of the Univ. of California, vi). University of California Press, Berkeley, 1922, p. 40.

of such structures by the repeated finding of the "placental sign" in a small group of pseudopregnant animals, held on dietary regimes lacking vitamin E. The sign, which consists in the appearance of blood in the vaginal canal at some time between the 13th and 15th days of pregnancy, had always been interpreted by us as leakage from the multiple new maternal vessels involved in placenta formation, and in our hands it had proven to be an invariable indication of preceding implantation and establishment of the placentae. It was all the more surprising, therefore, to find the "placental sign" in cases where we judged placentae could not have been formed.

Incidence of the "placental sign" in pseudopregnancy. A rather extensive study was now undertaken. Considerable numbers of young healthy females, which had been reared both on natural and on simplified food regimens, in the latter cases chiefly without vitamin E, were mated on the occurrence of oestrus with vasectomized males, and the inception of the condition of pseudopregnancy positively determined by the finding of plug fragments in the vaginal canal twelve or eighteen hours after placing the animals together. We now made careful daily microscopic examination of the cellular contents of the vaginal lumen and macroscopic examination with a small speculum. In this way we determined the time of earliest appearance of erythrocytes in the vaginal smear.

Table 1 exhibits the data secured. It may be summarized by the following statements: an extremely low proportion of pseudopregnant animals held on natural food regimens show the "placental sign" before the intervention of the next cycle (3.0 per cent). About half of the animals maintained on low E simplified food regimens show the sign, usually on the tenth or eleventh day, two or three days, therefore, before this sign is found in the case of true pregnancies of animals held upon natural food,[4] and, as it happens, also two or three days before the termination of their pseudopregnancy. That the much lower incidence of the "placental sign" with natural food regimens is really due to the presence of vitamin E in those regimens seems conclusively shown by the fact that merely adding twelve drops of wheat germ oil to the simplified ration decreased this incidence to that found with natural foods.

Incidence of spontaneous deciduomata in pseudopregnancy. We next sought to determine whether or not we were still dealing with a "placental sign," i.e., placental hemorrhage, in the cases of the vaginal blood in pseudopregnancy. Accordingly, a considerable number of animals, again both on natural food and E low dietaries, were bred with vasectomized males and after copulation, were retained for nine days, to be sacrificed on

[4] It is also a fact that in the true pregnancies of animals held on low E regimens pregnancies which, as we have shown, ultimately end in resorptions, the "placental sign" occurs somewhat earlier than normally.

the tenth day of pseudopregnancy. Table 2 shows that about 60 per cent of the animals on the E-free regimen showed one or more typical deciduomatous tumors, which had thus arisen spontaneously at one or more points along one or both uterine horns. It also shows that only about 4 per cent of the pseudopregnant animals held on natural food and autopsied on the same day showed such enlargements. This is in broad agreement with the results obtained in the case of the exhibition of the placental

TABLE 1

Incidence of the "placental sign"

Diet I. Whole wheat 67.5, casein 15, whole milk powder 10, sodium chloride 1, calcium carbonate 1.5, milk fat 5.

Diet 232. Casein 32, cornstarch (cooked) 40, salts no. 185 4, lard 22, cod liver oil 2

GROUP	NUMBER OF ANIMALS	NUMBER OF ANIMALS EXHIBITING "PLACENTAL SIGN"	ANIMALS EXHIBITING "PLACENTAL SIGN"	AVERAGE TIME OF APPEARANCE OF "PLACENTAL SIGN"	LENGTH OF PSEUDOPREGNANCY
			per cent	*days*	*days*
Natural foods:					
Table scrap diet—Group I	21	3	14.0	10.0	13.4
Table scrap diet—Group II	16	0	0	0	13.2
Diet I—Group I	47	0	0	0	15.1
Diet I—Group II	17	0	0	0	11.7
Total	101	3	3.0	10.0	13.3
Simplified diets (low vitamin E):					
Diet 232 +0.5 −0.7 gm. yeast—Group I	27	13	48.0		
Diet 232 +0.5 −0.7 gm. yeast—Group II	20	9	45.0	10.5	12.3
Diet 232 +0.5 −0.7 gm. yeast—Group III	28	17	61.0	12.1	14.0
Total	75	39	52.0	10.8	12.1
Simplified diets (+ high vitamin E):					
Diet 232 +0.5 −0.7 gm. yeast + 12 drops wheat germ oil	17	2	11.2	11.0	13.2
Diet 232 + 10 per cent fresh wheat germ	13	0	0	0	11.4
Total	30	2	6.7	11.0	12.3

sign in pseudopregnancy in the two groups of animals and would indicate that the deciduomatous structures constituted the sources of the hemorrhage. Confirmation of this conviction was secured in a series of 30 (table 3) cases, which were autopsied on or shortly after the day on which vaginal blood was detected. In every case deciduomata were present.

The inciting cause of the spontaneous deciduoma formation is enigmatical. The first thing that occurred to us was the possibility of unfertilized

eggs surviving long enough to descend through the oviduct, and thus represent the stimulating agent. In order to test this point, 16 animals were operated upon promptly after the occurrence of a positive mating and a section of the ovarian tip of each uterine horn, for a distance of some two millimeters, removed between the ligatures. All doubt that the continuity between the oviduct and uterus had been destroyed was removed by the examination of several sets of serial sections which all showed

TABLE 2

Incidence of spontaneous deciduomata

Diet I. Whole wheat 67.5, casein 15, whole milk powder 10, sodium chloride 1, calcium carbonate 1.5, milk fat 5.

Diet 232. Casein 32, cornstarch (cooked) 40, salts no. 185 4, lard 22, cod liver oil 2.

GROUP	NUMBER OF ANIMALS AUTOPSIED	TIME OF AUTOPSY—DAY OF PREGNANCY	NUMBER OF ANIMALS EXHIBITING DECIDUOMATA	ANIMALS EXHIBITING DECIDUOMATA
				per cent
Natural foods:				
Diet I—Group I	18	9–12	0	0
Diet I—Group II	9	10	1	11.0
Total	27		1	3.7
Simplified diets (low vitamin E):				
Diet 232 +0.5 −0.7 gm. yeast—Group I	24	10–16	17	71.0
Diet 232 +0.5 −0.7 gm. yeast—Group II	18	10	9	50.0
Diet 232 +0.5 −0.7 gm. yeast—Group III	20	10	12	60.0
Total	62		38	61.0
Simplified diets (+ high vitamin E):				
Diet 232 +0.5 −0.7 gm. yeast + 12 drops wheat germ oil—Group I	21	10	2	9.5
Diet 232 +0.5 −0.7 gm. yeast + 12 drops wheat germ oil—Group II	20	10	0	0
Diet 232 +0.5 −0.7 gm. yeast + 12 drops wheat germ oil—Group III	16	10–12	1	6.0
Total	57		3	5.3

obliteration of the lumen and in fact absence of epithelial tissue in the hiatus. The animals were sacrificed on the tenth day. The incidence of deciduomatous tumors in the two groups was either the same or considerably higher than we had hitherto encountered (56 per cent to 86 per cent). Unfertilized eggs are hence not the stimulating agents.

Characteristics of the deciduomatous tumors. The spontaneous deciduomatous tumors here dealt with were in most cases spherical enlargements

of the uterine horn, resembling strikingly the reaction about a thread or other minute foreign body, or in fact the enlargements at about the 12th or later days of pregnancy, save for the omission of fetal elements. Sections show that the spontaneous tumors are constituted by the transformed connective tissue of the endometrium.[5]

It is highly interesting that occasionally the deciduomatous response is diffuse and characterizes a larger portion of the uterine horn.

TABLE 3

Relation of the "placental sign" to the presence of deciduomata

Diet 232. Casein 32, cornstarch (cooked) 40, salts no. 185 4, lard 22, cod liver oil 2

GROUP	NUMBER OF ANIMALS AUTOPSIED AFTER EXHIBITING THE "PLACENTAL SIGN"	NUMBER OF ANIMALS EXHIBITING DECIDUOMATA	PER CENT OF ANIMALS EXHIBITING DECIDUOMATA
Diet 232 +0.5 −0.7 gm. yeast Group I	13	13	100.0
Diet 232 +0.5 −0.7 gm. yeast Group II	9	9	100.0
Diet 232 +0.5 −0.7 gm. yeast Group III	8	8	100.0
Total	30	30	100.0

We have given some thought to the hypothesis of points of mild infection or inflammation constituting the inciting cause of the tumors. Pus pockets are sometimes found in the tumors. Should we maintain this view, it would be necessary to suppose that the chief cause for higher incidence of the tumors in low E was due to the less healthy state of the endometrium. But that there is a more direct relation of deciduoma growth to low E would seem to be shown by the fact that when tumors are purposely produced under E high and low E conditions by the insertion of threads, the tumors clearly tend to be larger with impoverished E.

All our evidence points to the peculiar ease with which deciduomatous response can be evoked with impoverishment in E, an ease so marked that these tumors grow spontaneously in one or both uterine horns in a high proportion of E low females bred with vasectomized males.

[5] Dr. Lucien Brouha has recently demonstrated in this laboratory that a localized hypertrophy and overfunction of the muscularis also occurs, sharply delimited at these particular points.

STERILITY IN INBRED RATS[1]

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In 1919 King[2] as a result of the most thoroughgoing study ever conducted with mammals believed she had adduced evidence to show that if selection is made for constitutional vigor very extensive inbreeding need not alter the fertility of mammals. These data were secured by the study of twenty-five inbred generations (brother-sister matings) involving well over three thousand litters. In December 1923, through the kindness of Doctor King, Professor S. J. Holmes of this University received several members (not brothers and sisters) of the forty-ninth generation of one of the series (B) of the same strain upon which the extensive King reports have been made. Brother-sister matings were allowed to occur in the case of the animals in Professor Holmes' hands and we obtained some of the young adults of the fifty-fifth generation. We have conducted exclusively brother-sister matings and have studied with some care the growth and sexual physiology of from fifteen to thirty-seven males and females each of the fifty-sixth, fifty-seventh and fifty-eighth generations. No selection has been exercised by us,[3] so that the behavior of this stock reported herewith need not be taken as invalidating the King statement that inbreeding *with* selection need not produce sterility and other deleterious effects.

Figure 1 depicts the average growth during the first three months of life of males and females belonging to the fifty-sixth, fifty-seventh and fifty-eighth generation respectively. The curve may indicate a slight inferiority of growth of the fifty-eighth generation, but the numbers taken for experimentation were doubtless too low to enable us to make a satisfactory statistical statement. In the case of the fifty-sixth generation the

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and from the Bureau of Dairying of the United States Department of Agriculture. These funds have been generously augmented by the Board of Research and by the College of Agriculture of this institution.

[2] King, Helen Dean. Studies on inbreeding. Wistar Inst. of Anatomy and Biology, Philadelphia, 1919, 8°, 175 p.

[3] On the day of birth of a litter its number, in case it exceeded six, was reduced to six and an effort was made so far as possible to have this comprised of four females and two males. The animals retained for lactation were always the heavier individuals of each sex.

growth during the first three months of life was compared in the case of both males and females with the same sized group of animals from our hybrid albinous Norwegian strain. Care was taken to give the same nutritional regime to both strains (our stock ration—Diet I: whole wheat 67.5, casein 15, whole milk powder 10, NaCl 1, $CaCO_3$ 1.5,milkf at 5, with fresh lettuce). Comparison shows that the King inbred albinous strain grows almost as well as our hybrid strain, the growth and stature being superior to that of any albinous strain known to us.[4]

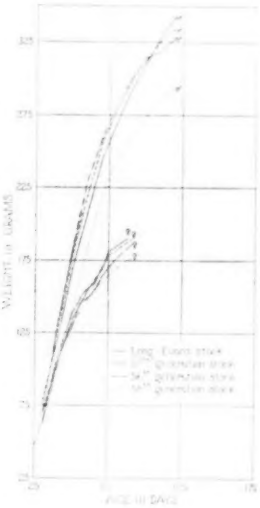

Fig. 1

Table 1 shows the maturity and succession of ovulation cycles characterizing the early life of females belonging to the three generations here reported. For comparison, we give data for our normal hybrid strain, data secured from the study of a very large number of individuals. It will be seen at once that the inbred albinos mature somewhat earlier and have a superior ovulation rhythm when compared with the more vigorous and longer-lived animals of our hybrid strain—a rather surprising fact.

No attempt at breeding animals was made until the completion of the nintieth day of life. It was a rule after this date to attempt to mate every female at the occurrence of pro-oestrus as determined by the vaginal smear. She was placed in the cage inhabited by either of her two brothers and a search made the following morning for the presence of the vaginal plug or of residual spermatozoa. Almost as soon as the work was begun we encountered a surprising number of "refused" matings under these conditions, i.e., evidently copulation had not occurred.[5] The proportion of such failures to copulate in our own stock colony is well known to us from the study of several thousand attempted matings. About 10 per cent of

[4] We have studied the standard Wistar and Slonaker albinous strains. It is only fair to state, however, that the excellent early condition and growth of these animals is not succeeded by a correspondingly excellent later performance. In spite of the best dietary care, the animals become poorly nourished or emaciated in appearance, have parasitic diseases of the skin and rapidly succumb from intercurrent infections so that their longevity was conspicuously less than that of our stock.

[5] That such a result was not due to the impairment of the ejaculatory mechanism, for instance, but to inadequacy of sex interest was shown by actual two-hour observations of such attempted matings and similar pairings of the Helen Dean King animals with normal sexually active partners of our own stock.

the cases in which a young, vigorous female known by the method of vaginal smear to be in the pro-oestrous stage is mated with an equally vigorous male resulted in "refusals" as shown by the study of the vaginal contents fifteen hours afterwards. Table 2 will show that from 62 to 76 per cent of these brother-sister matings of the King inbred stock resulted in no copulation, a thing occurring in only 20 per cent of normal cases. It is impossible from such data to know whether this anomaly of behavior

TABLE 1

GROUP	NUMBER OF ANIMALS IN GROUP	AVERAGE AGE AT FIRST OESTRUS	AVERAGE LENGTH OF CYCLES	AVERAGE WEIGHT AT 90 DAYS
		days	*days*	*grams*
Helen Dean King Stock, 56th generation	37	36.0	4.7	185
Helen Dean King Stock 57th generation	31	37.4	4.9	189
Helen Dean King Stock, 58th generation	19	39.1	4.3	176
Long-Evans Stock	*153*	*44.8*	*5.5*	*190*

TABLE 2

GROUP	NUMBER OF INSTANCES IN WHICH BROTHER AND SISTER WERE PLACED TOGETHER WHEN FEMALE WAS IN OESTRUS	NUMBER OF INSTANCES IN WHICH COPULATION OCCURRED	PERCENTAGE OF CASES IN WHICH COPULATION OCCURRED	NUMBER OF IMPLANTATIONS RESULTING FROM COPULATION	PERCENTAGE OF IMPLANTATIONS RESULTING FROM THESE COPULATIONS	NUMBER OF LITTERS PRODUCED	PERCENTAGE OF IMPLANTATIONS RESULTING IN A LITTER
Helen Dean King Stock, 56th generation	109	38	35.0	29	76.0	28	96.0
Helen Dean King Stock, 57th generation	111	32	29.0	26	81.0	26	100.0
Helen Dean King Stock, 58th generation	42	10	24.0	10	100.0	10	100.0
Long-Evans Stock	*1,777*	*1,416*	*80.0*				
		909		*840*	*92.0*	*832*	*99.0*

affected solely or occurred to a predominant extent in one sex. In order to shed more light on this we mated both males and females of the King inbred strain with normal active individuals of our strain after several attempted brother-sister matings had failed. In some instances the attempted union with our stock was successful; in more instances this was still not the case. There were, however, always more copulations resulting from these attempted matings than was the case with a similar number of brother-sister matings, indicating delinquency in both male and female

of the King inbred strain. Further, the indisposition to copulate was exhibited to a greater extent by males than by females of the inbred strain.

Proportion of conceptions. Table 2 shows that in the successive generations 76 per cent, 81 per cent and 100 per cent of the definite copulations resulted in implantation as detected by the erythrocyte sign. The last summary of the data from our own stock colony gives an average implantation per cent of 92 $\frac{(840)}{909}$. The inbred animals are not, therefore, very seriously disturbed in their power to conceive, a fact in striking contrast with their indisposition to mate. Clearly, most if not all of the interference with reproduction—sterility—in these animals is due to impairment of sex behavior and but little, if any, of it to defect in the germ cells. Whether or not further deterioration would involve germ cell impairment is, of course, entirely unknown to us. We can merely record our rather extensive analysis as showing a somewhat remarkable behavior or hormonal cause of the relative sterility of this particular strain of inbred animals.

CUTANEOUS RESPIRATION OF THE CAT

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Krogh's (1904) experiments on the cutaneous respiration of the pigeon, tortoise, frog and eel showed that carbon dioxide is eliminated through the skin and that oxygen is absorbed. He concluded that oxygen absorption is a function of the permeability of the epidermis, the closeness of the capillary bed and the difference of tension of the gases on the two sides of the skin membrane. In the eel, these factors combined to render the exchange of gases through the skin so active as to enable it to live without the use of its gills. At the other extreme the exchange of gases through the heavy coat of the tortoise was almost nil.

Experiments of the same type on mammals appear to be quite lacking. As regards human skin it has long been known that carbon dioxide is given off and that oxygen is absorbed, but this work was done before the modern methods of gas analysis had been perfected and may be looked upon as qualitative rather than quantitative in its value. Furthermore, there has been no attempt to correlate the oxygen absorption with the carbon dioxide output.

In this paper it is shown that there is a measurable metabolism of the skin of the cat but that the diffusion of carbon dioxide and oxygen through the skin, as indicated in Krogh's experiments, is highly improbable. The cutaneous respiration of the human skin is being studied at the present time in this laboratory.

METHOD. A cat was given an intraperitoneal injection of barbital-sodium, 0.3 gram per kilogram of body weight. The body was then enclosed in a gas-tight plethysmograph from which samples of gas were withdrawn at intervals and the carbon dioxide and oxygen content analysed in a Haldane apparatus. The duration of the experiment was from two to five hours. Gas samples were withdrawn every hour from the plethysmograph directly into gas collecting tubes. The change in percentage composition of the carbon dioxide and oxygen multiplied by the total volume of gas in contact with the body gives the volume of carbon dioxide and oxygen which has been lost or gained over a given interval.

The plethysmograph was made of iron with a removable lid which fitted

into a mercury seal (fig. 1). Owing to the solvent action of mercury on solder it was necessary that the plethysmograph should be cast. A water seal was not permissible owing to the readiness with which carbon dioxide passes through it.

An opening was left at one end of the plethysmograph over which a rubber dam 1 mm. in thickness was stretched. A hole was then cut in the dam of such dimensions as to close it tightly about the neck after the head of the cat had been drawn through. Owing to the difficulty of stretching and fastening the dam properly over the aperture for the head, the collar to which the dam was attached was made removable in the

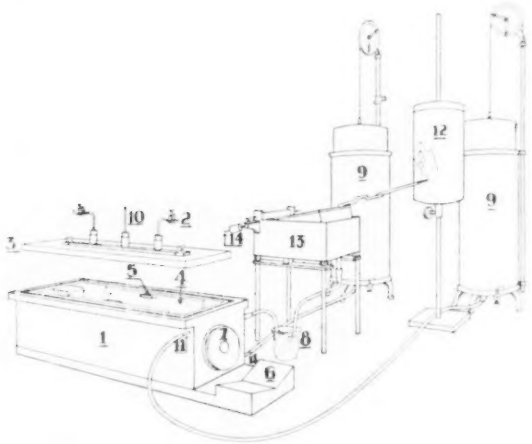

Fig. 1. Apparatus for determining cutaneous respiration. *1*, plethysmograph; *2*, gas sampling tube; *3*, cover; *4*, blocks to reduce space; *5*, cleats; *6*, head rest; *7*, head aperture; *8*, condensation chamber; *9*, mixing spirometer; *10*, thermometer; *11*, tubes entering gas seal; *12*, kymograph; *13*, Krogh spirometer; *14*, counterbalancing pin.

form of a brass ring (fig. 2). This was threaded so that it screwed directly into the head aperture which was likewise threaded to receive the collar. In order to fasten the dam to the collar the latter was removed from the plethysmograph and screwed into a cylinder about 4 inches long which stood upon its end. While the collar was thus held in a convenient position the dam was stretched over it and held fast by a wire (soft iron, 2 mm. in diameter) which fitted into the groove around the rim of the collar. A hole (about 3 cm. in diameter for a cat weighing 3 kilos) was then cut in the dam with curved scissors.

As a preventive against leaks which would otherwise take place between

the threaded junction of the collar and the head aperture the collar was fitted with a washer cut out of sheet rubber (5 mm. in thickness). A heavy coating of vaseline was then applied to the threads of the collar and also to the surface of the washer which came in contact with the plethysmograph. In order to make the contact between the rubber dam and the neck air-tight, the neck of the cat was shaved and a coat of vaseline applied. The hole in the rubber dam was made smaller than the neck of the animal causing the edge to be slightly reflected and thus bringing a relatively broad surface of rubber to bear upon the skin. Another coat of

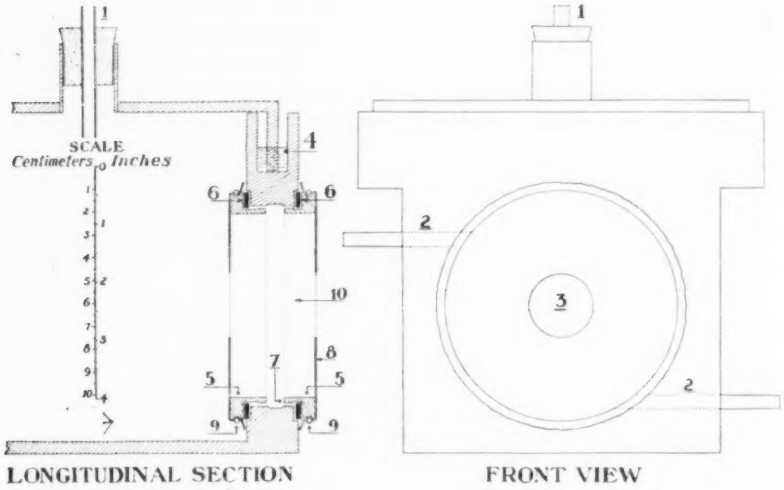

Fig. 2. Detailed drawing of plethysmograph. *1*, gas collecting tube; *2*, tubes entering gas seal; *3*, head aperture through rubber dam; *4*, mercury seal; *5*, brass collar for attachment of rubber dam; *6*, rubber washer; *7*, threaded joint between brass collar and plethysmograph; *8*, rubber dam; *9*, wire binding rubber dam to brass collar; *10*, gas seal.

vaseline was applied to the reflected edge of the collar after the neck was in place.

When the plethysmograph was filled with room air an inward leak was of no consequence. On the other hand, when the plethysmograph was filled with gas having a composition different from air, a very small inward leak would invalidate the results. If, for example, the plethysmograph were filled with nitrogen, a slight leak of air into the plethysmograph would obviously give misleading results with respect to the oxygen which, under these circumstances, would pass from the blood into the gas surrounding the body. To avoid such a situation a second dam, in every respect simi-

lar to that just described, was applied to the plethysmograph in such a manner as to constitute a gaseous seal interposed between the air and the gases in the plethysmograph (fig. 2). Through the space that intervened between the two dams a gas of the same composition as that in the plethysmograph was kept in circulation by passing it from one spirometer (capacity 5 liters) to another. The gas seal communicated with the spirometers by means of holes drilled through the heavy wall of the plethysmograph. If, then, a small quantity of air is passed through or around the outer dam it at once became so diluted by admixture with the gas in the seal that the composition of the latter was still essentially unaffected. The effect of a leak through the inner dam was thus reduced to an extent which

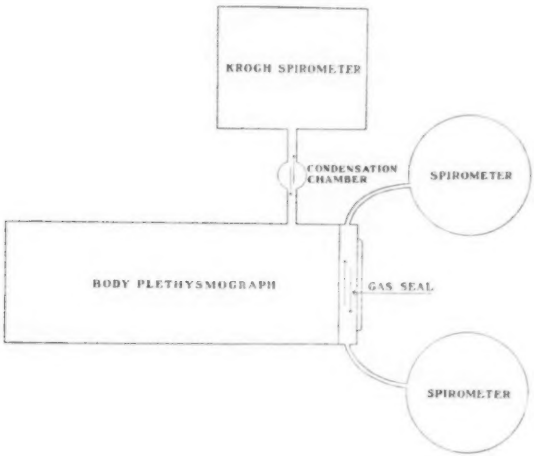

Fig. 3

was negligible. Under the circumstances of such an experiment there is also a tendency for the gases to diffuse through the rubber dam. This tendency is greatly minimized, however, by the presence of the gas seal.

It may be contended that all these precautions, taken to prevent the gas leaks which are involved by the head protruding through the end of the plethysmograph, could have been obviated by placing the entire animal in a plethysmograph. Under these conditions the breathing could be accomplished through a tube leading from a tracheal cannula to the room air. There are, however, two objections to this method: 1, the possibility of some expired air escaping into the plethysmograph; and 2, the impossibility of preventing the exchange of gases through the mucous membrane of the mouth.

The pressure of the gas within the plethysmograph had to be kept equal to the outside pressure or the danger from leaks became iminent. The volume of air surrounding the body of the cat in the plethysmograph was about 3500 cc., of which 600 cc. were withdrawn during the experiment for analysis. In order to keep the gas in the plethysmograph at atmospheric pressure, a Krogh spirometer was connected to the plethysmograph to serve as a reservoir from which gas samples could be withdrawn. This spirometer (1 liter in capacity) was operated in a mercury seal to prevent loss of carbon dioxide. It was cast in iron with a light sheet-iron lid, all the seams being made air-tight by a heavy coating of shellac. Solder could not be used for reasons already explained. The volume of gas in the spirometer was indicated by the distance of the writing point above the base line as indicated on the smoked surface of the kymograph sheet. The position of the writing point also served as a means of detecting the presence of a leak. Providing the temperature within the plethysmograph was constant, the writing point remained within the limits of the oscillations caused by respiratory movements. A slow rise or fall of its position indicated a leak.[1]

A glass condensation chamber, inserted in the tube connecting the plethysmograph with the Krogh spirometer and immersed in a beaker of ice water, served to condense out the water vapor of the air which passed through it as it was pumped in and out of the spirometer by the respira-

[1] The apparatus here described is, with certain additions and modifications, an adaptation of a plethysmograph which the author has had in use since 1924 for recording both respiratory movements and respiratory volumes. When used for this purpose the plethysmograph should be large enough to contain a board to which the animal may be properly tied at full length. Only the inner dam is necessary. The condensation chamber may be omitted and the plethysmograph connected directly to the spirometer by tubing of large bore to minimize the resistance. A Krogh spirometer having a water rather than a mercury seal should be used since water will impose less resistance to movements of the lid than mercury. Slight leaks about the neck are of no consequence. If the spirometer is properly counterbalanced it will continue to write upon whatever level it is set. Leaks of more serious nature, however, will cause a progressive rise or fall of the writing level, thus rendering a continuous record impossible.

Since the same volume of air that is drawn into the lungs must be displaced from the plethysmograph by the expansion of the chest, and *vice versa* upon expiration, the movement of the spirometer writes a record on the kymograph which is quantitative as well as qualitative. The slightest irregularity in breathing will be recorded. If desired, the minute volume of respiration may be recorded automatically by attaching a counter (Roy and Adami, 1892) to the counterbalancing pin (fig. 1) of the spirometer at any convenient point, thus adding mechanically the movement of the spirometer lid. When using the Roy and Adami counter for such a purpose, parts *A*, *C* and *P* of their apparatus are omitted, and the lever *B* may be attached directly by a silk thread to the counterbalancing pin of the spirometer lid. The lever will then rise as the spirometer lid falls, while a weight suspended from the lever will pull it down as the spirometer lid rises.

tion movements. An excessive accumulation of moisture in the air surrounding the body was thus prevented.

The accuracy of the determination of the carbon dioxide and oxygen which pass through the skin increases as the volume of gas in the system (plethysmograph and spirometer) decreases. It was desirable, therefore, that the extra-bodily space should be reduced as far as possible. To this end, blocks of wood were cut and modelled to fit the contour of the body, so that when a cat weighing 3 kilograms was in the plethysmograph the volume of extra-bodily gas did not exceed 4 liters (3 liters in the plethysmograph and 1 liter in the spirometer). Cleats attached to these blocks held fast the cords fastened about the legs of the cat to prevent it from

TABLE 1

*Exchange of CO_2 and O_2 through the skin when the body is surrounded by air of normal composition**

EXPERIMENT NUMBER	WEIGHT OF CAT	TEMPERATURE	PERIOD	CO_2 EXCRETED	O_2 ABSORBED	R.Q.
	kilos	*°C.*	*hours*	*cc. per hour*	*cc. per hour*	
2	2.7		2	8.56	12.79	0.67
3	2.7		2	6.30	7.21	0.87
4	3.3		2	6.76	7.66	0.88
5	2.4	30.0	2	7.62	6.23	1.22
6	3.0	30.0	3	5.25	5.35	0.98
7	2.8	30.0	5	5.68	3.94	1.44
8	2.5	29.0	5	2.60	3.12	0.83
9	2.8	29.0	5	3.50	2.68	1.31
10	2.5	28.0	5	5.80	8.02	0.72
12	2.6	28.0	5	3.33	2.29	1.44
13	2.8	33.5	4	6.01	6.51	0.92
15	2.5	31.0	3	4.81	5.18	0.93
Average..	2.71			5.52	5.92	1.02

* Volumes reduced to standard conditions of temperature and pressure.

shifting its position in the plethysmograph. The analytical error for the determination of carbon dioxide in this system was 0.4 cc., and for the determination of oxygen 0.8 cc.

EXPERIMENTAL RESULTS. *Exchange of carbon dioxide and oxygen between the skin and air of normal composition.* The average rate of carbon dioxide excretion by the skin of a cat weighing 2.7 kilos is 5.5 cc. per hour, with an average rate of oxygen absorption of 5.9 cc. per hour (table 1). Although there is a wide individual difference in the rate of cutaneous respiration, the ratio of the carbon dioxide excreted to the oxygen absorbed falls, with the exception of four cases, within the physiological range of a true respiratory quotient, allowance being made for experimental errors.

Exchange of carbon dioxide and oxygen between the skin and air having a high carbon dioxide and a low oxygen content. In these experiments the normal conditions have been reversed, i.e., the oxygen tension of the surrounding air was lower than that of the body fluids and the carbon dioxide tension higher. If the carbon dioxide tension of the air were higher than that of the blood we should expect to find carbon dioxide passing from the air into the body; and if the oxygen tension of the air were lower than that of the blood we should expect oxygen to pass from the body into the air. This, in fact, is what happened.

Since the rate at which carbon dioxide and oxygen pass through the skin under these circumstances is proportional to the tension difference existing between the gases of the cutaneous blood and the gases which surround the

TABLE 2

Exchange of CO_2 and O_2 through the skin when the body is surrounded by a high CO_2 and a low O_2 tension

	EXPERIMENT NUMBER	TENSION		VOLUME OF GAS	
		At start	At end first hour	First hour	Per hour per millimeter pressure difference
		mm. Hg	*mm.* Hg	*cc.*	*cc.*
				Absorbed	
Carbon dioxide	11	50.4	43.6	30.8	5.7
	14	85.7	70.5	68.8	1.69
	16	69.2	58.6	44.6	1.84
				Excreted	
Oxygen	11	24.9	40.2	78.4	0.97
	14	19.9	41.5	96.8	1.12
	16	44.9	58.7	62.9	1.03

body, any computation of the rate must be based upon these differences. It is probably fair to assume that the tension of carbon dioxide in the cutaneous blood is about 45 mm. of mercury. It is not so easy, however, to arrive at a figure for the oxygen tension. The experiments under discussion were continued over a period of five hours, and at their termination it was found that the oxygen concentration of the gas surrounding the body was steadily mounting, having already reached in one instance a tension of 72 mm. For this reason I am inclined to believe that the oxygen tension of the cutaneous blood is nearer to that of the arterial than of the venous blood. I shall, therefore, take 106 mm. as the tension of oxygen in the cutaneous blood for the purpose of making my computations.

In table 2 are presented the data on three experiments in which the body

was surrounded by a gas having a carbon dioxide tension higher than that of the blood and an oxygen tension which was lower. The comparative rates at which carbon dioxide and oxygen pass through the skin were computed in terms of cubic centimeters absorbed or excreted over a period of one hour when the pressure difference between the blood gases and the gases surrounding the body was equal to 1 mm. of mercury. Let us take, for example, experiment 14 and see how this value for the carbon dioxide absorption has been derived. The tension of carbon dioxide in the plethysmograph at the beginning of the experiment was 85.7 mm. and fell to 70.5 mm. at the end of the first hour, indicating a carbon dioxide absorption of 68.8 cc. during this period. If we take 45 mm. as the carbon dioxide tension in the blood, the pressure difference between the carbon dioxide of the blood and that of the gas in contact with the skin is (85.7 to 45.0) 40.7 mm. Since the diffusion rate of a gas through a membrane is proportional to its partial pressure, the volume of carbon dioxide that would pass through the skin in 1 hour under a pressure difference of 1 mm. would be 68.8 cc. divided by 40.7 or 1.69 cc. The rates for oxygen diffusion have been derived in the same manner. Taking 106 mm. as the oxygen tension of the cutaneous blood, the rate of oxygen diffusion was 1.04 cc. per hour under a pressure difference of 1 mm. If we take 76 mm. as the oxygen tension of the cutaneous blood, the rate of oxygen diffusion would be 1.76 cc. per hour per millimeter pressure difference.

Let us now compute the rate at which carbon dioxide would be excreted by the skin, assuming the body to be surrounded by room air and the outward rate of carbon dioxide diffusion to be equal to the inward rate. For the rate of inward diffusion we may take as a conservative figure the average of experiments 14 and 16 (table 2), which is 1.77 cc. per hour per millimeter pressure difference. If the carbon dioxide pressure difference between the cutaneous blood and the air is 45 mm., then carbon dioxide should pass through the skin of the cat at the rate of (45 × 1.77) 79.6 cc. per hour. In the same manner we may compute the rate at which oxygen would be absorbed through the skin when the body is surrounded by room air, assuming the inward rate of oxygen diffusion to be equal to the outward rate. With an oxygen tension of cutaneous blood of 106 mm. there would be a pressure difference of (159 − 106) 53 mm. between the blood and the air, which, under the conditions postulated, would cause an hourly absorption of (53 × 1.04) 55.1 cc. of oxygen through the cat's skin.

The excretion of carbon dioxide by the skin when the carbon dioxide tension of the body fluids has been raised. If the carbon dioxide which is given off by the body under normal conditions is a function of the carbon dioxide tension of the blood in the cutaneous capillaries, then we should expect that if the tension of carbon dioxide in the blood were increased we should find a proportionate increase in the rate of carbon dioxide excretion through

the skin. The carbon dioxide tension of the blood may be raised by permitting the cat to breathe a gas mixture of any desired carbon dioxide concentration through Tissot valves connected to a tracheal cannula. Three experiments were made using 7.2, 7.2 and 10.0 per cent carbon dioxide respectively in the inspired gas mixtures. In the first two experiments the cat received artificial ventilation under curare so that the rate of ventilation could be held constant. In these cases the carbon dioxide tension of the inspired air must be added to the already existing carbon dioxide tension in the alveoli, since the respiratory volume per minute remained unchanged. In the third experiment the cat was allowed to breathe naturally, and it is necessary to take into account the effect which the accelerated respiration, due to the carbon dioxide stimulus, has in reducing the alveolar carbon dioxide tension. In the first two experiments the alveolar carbon dioxide tension was, therefore, increased about 120 per cent, and in the third about 90 per cent. For a period of two hours the cat was allowed to breathe room air, and then followed a period of two hours during which the carbon dioxide mixture was administered. In every case it was found that the rate of carbon dioxide excretion through the skin during the inhalation of the carbon dioxide gas mixture was identical with the rate of excretion during the inhalation of room air. If the carbon dioxide which is given off by the skin when surrounded by room air had its origin in the blood, then we should expect that by doubling the carbon dioxide tension of the blood we should double the carbon dioxide output. The carbon dioxide output, however, remained unchanged under these circumstances. We must conclude from this experiment that the skin is impermeable to the outward diffusion of carbon dioxide; and since the epidermis is all that intervenes between the capillary loops of the skin and the air, it is due to this particular layer of the skin alone that the loss of carbon dioxide from the blood is so effectually restrained.

Discussion. The oxygen absorption through the lungs of a cat weighing 2.7 kilograms is about 1100 cc. per hour, whereas the oxygen absorption through the skin per hour is only 5.9 cc. The cutaneous respiration is, therefore, about 0.54 per cent of the pulmonary respiration. The oxygen absorption per unit of body surface is 31.4 cc. per square meter. The area of body surface was deduced from direct measurements. After the skin was removed from the body it was laid over a sheet of heavy paper, upon which the skin pattern was traced. The pattern was then cut out and weighed, the area being proportionate to the weight.

Since the skin is impermeable to the outward diffusion of carbon dioxide, the carbon dioxide excreted must have its origin in the epidermis itself as a result of oxidative processes going on in that tissue. Such a conclusion receives still further support from the fact that the ratio of the carbon dioxide excreted to the oxygen absorbed fell, in most cases, within the

limits of a respiratory quotient. In a like manner we may conclude that the skin is impermeable to the passage of oxygen from the air into the blood. The oxygen which has been absorbed by the skin has, in reality, gone no further than the epidermis, where it has been engaged in effecting metabolic processes of a wholly independent nature.

Thus the skin, through the agency of the epidermis, is seen to act as a very effective barrier against any possible disturbance of the blood gases by contact with the air. This barrier may be of questionable value with respect to the inward diffusion of oxygen, but with respect to the outward diffusion of carbon dioxide it unquestionably serves to maintain the proper acid-base base balance in the cutaneous blood. That the skin should be freely permeable to the passage of carbon dioxide and oxygen when the normal direction in which these gases pass has been reversed is an interesting fact, but wholly unexplainable upon teleological grounds.

CONCLUSIONS

1. The cutaneous respiration of the cat is about 0.54 per cent of the pulmonary respiration. The oxygen absorption in terms of absorption per unit of body surface is 31.4 cc. per square meter.

2. The exchange of gases through the skin under normal conditions is the result of wholly independent metabolic processes which are confined to the epidermis. The deeper layers of the skin are not involved.

3. The skin exhibits an irreciprocal permeability to the passage through it of carbon dioxide and oxygen. We have reason to believe that the skin is impermeable to the outward diffusion of carbon dioxide and to the inward diffusion of oxygen; whereas it is freely permeable to carbon dioxide and oxygen when passing in the reverse directions.

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